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Research report

NF-κB p50-deficient mice show reduced anxiety-like behaviors in tests of exploratory drive and anxiety

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Abstract

The ubiquitous transcription factor nuclear factor (NF)-κB plays a prominent role in regulation of inflammatory immune responses and in cell survival. Recently, it has been found to be active in neurons, and mice lacking NF-κB subunits p50 or p65 show deficits in specific cognitive tasks. Here we demonstrate a strikingly low level of anxiety-like behavior in the $p50^{-/-}$ mouse. In an open field, the mutant mice showed significantly less defecation, more rearing, and more time spent in the center compartment relative to wild type control mice. The $p50^{-/-}$ mice also spent more time investigating a novel object placed in the open field. On the elevated plus maze, $p50^{-/-}$ mice spent more time on the open arms and had increased numbers of open arm entries relative to wild type. In group housing conditions, they did not establish dominant–subordinate hierarchies, whereas wild type control animals did so, in part, by whisker barbering and conspecific allogrooming. In tests of general health, sensorimotor function, and daily activity on a circadian rhythm, $p50^{-/-}$ mice were normal. Thus, absence of the p50 subunit of the NF-κB transcription factor, which results in altered NF-κB transcriptional activity in cells throughout the body and brain, alters neuronal circuitry underlying manifestation of emotional behavior. The p50 subunit appears to play a role in normal expression of certain forms of anxiety.

Keywords: Anxiety; Fear; NF-κB; Knockout mouse; Transcription factor; Immune

1. Introduction

The NF-κB transcription factor is present in all cells and organ systems and is traditionally associated with immune system function. Physical stress and pathogens trigger its translocation from cytoplasm to nucleus where it regulates the transcription of genes affecting inflammatory responses and cell survival pathways [6,28]. NF-κB comprises two subunits, usually p65 and p50, that are constrained in the cytoplasm by an inhibitory subunit IκB, which has several forms [29]. The subunits are members of the larger NF-κB/Rel family of transcription factors that include p50 (cleaved from its precursor p105), p52 (cleaved from p100), p65 (RelA), c-Rel, and RelB. All five proteins can form homo- and heterodimers that bind to cognate κB DNA binding sequences to regulate transcription.

The role of NF-κB in neuronal function and CNS processes that control higher order behavior is only beginning to be researched. One potential mechanism of influence is indirect, via peripheral immune modification that can then alter CNS function. More recently, direct NF-κB involvement in CNS functions has been implied. Constitutive neuronal NF-κB activity has been documented [21]. Transgenic mice with reporter genes for NF-κB activity show widespread NF-κB function in neurons in adult brain, notably in the hippocampus, cerebral cortex, amygdala, hypothalamus, and discrete brainstem nuclei [11,43]. In several measured locations (cerebrum, hippocampus, pons, and hypothalamus), the identified NF-κB complexes were primarily p50/p65 heterodimers and also p50/p50 homodimers [5,43].

Neuronal NF-κB activity has been linked to neuroprotection [17]. NF-κB has been also been associated with CNS processes involving learning and memory [16,51]. NF-κB and its regulated immune system signal molecules such as IL-1β and TNF-α have all been shown to be involved in long-term potentiation (LTP) and hippocampal-dependent learning [1,8,40]. Very recently, NF-κB was localized to neuronal synapses and shown to participate in synaptic signaling

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and hippocampal-dependent learning [31]. Neuronal NF-κB activity can be triggered by glutamate [20] and synaptic input in vitro [31]. However, little is known about how these observations relate to functional circuits in vivo. One study demonstrated involvement of an NF-κB-dependent mechanism in the amygdala in fear conditioning [51].

Additional advances in this regard have been made by observations of animals with NF-κB subunits knocked out. Baltimore's group systematically deleted the NF-κB subunits in mice to examine their roles in vivo. Deletion of the p65 subunit is embryonically lethal [9], but the knockout can be rescued by concurrent deletion of type 1 TNF receptor [2]. The p65/TNFR1 knockout mice are infection-prone, but their brains appear normal by gross examination, and there are no obvious behavioral abnormalities. However, selective deficits were noted in spatial radial arm maze performance [31].

NFKB1 (p50) gene-deleted mice are viable and fertile. They have no obvious developmental or histopathological abnormalities, but they do display selective defects in B cell responses and in basal and specific antibody production [45,48,52]. Non-specific responses to infection are affected in p50 knockout (KO) mice, with reduced immunoglobulin secretion and class switching [45]. The p50 KO mouse has been examined in models of neuronal degeneration. An increased vulnerability to toxic insult in these mice suggests that the normal presence of p50 is neuroprotective [52,53]. In addition, p50 KO mice perform poorly in acquiring escape behavior in an active avoidance paradigm [22] and fail to display acupuncture-induced analgesia [35], suggesting roles for the p50 subunit (and therefore the NF-κB transcription factor) in learning, attention, and motivated behavior. Although a role for NF-κB has been shown in learning of emotional responses [51], anxiety-like behavior has never been formally characterized in mice lacking NF-κB p50.

Anxiety is a higher order physiological and psychological response to perceived threats. Many human mood disorders are characterized by excessive anxiety [34]. Several studies have explored the interrelationship of anxiety, CNS stress circuitry involving the hypothalamic–pituitary–adrenal (HPA) axis, and immune system responses [41]. Most studies emphasize the effects of anxiety (or stress) on immune function, but others suggest that immune system alterations (elevated cytokine levels) can produce symptoms resembling depression [30]. Current evidence suggests that anxiety levels are regulated by genetic factors [15,47], though as of yet, there is only scant evidence that immune signaling genes are linked to mood states [18,32].

Emotional states in animals are inferred on the basis of behavioral tests demonstrated to have face or predictive validity [49], and the neurochemical, physiological, and neuroanatomical bases of such states are only partially understood. One approach to understanding the biochemical basis of emotion is to study behaviors in transgenic or knockout animals to gain insights about the involvement of individual gene products in influencing neural systems subserving emotional behaviors. Many candidate genes have been examined, and others have been serendipitously discovered to be associated with emotional behavior [46], including some immune signal genes [33,50]. Transcription factors are a major target for learning and memory studies, but they have not been prominent in emotion research. The present work provides evidence that NF-κB signaling is a key component of manifestation of anxiety-related behavior. In addition to standard tests of anxiety-like behavior, the animals were tested for related behaviors: sensorimotor responses, overall activity levels, response to novelty, and establishment of dominant–subordinate hierarchies.

2. Materials and methods

2.1. Mice

Two-month-old male $p50^{-/-}$ (KO) and wild type (WT) control mice (B6; 129P2-Nfkb1^{tm1Bal} and B6129PF1, respectively) were purchased from The Jackson Laboratory (Bar Harbor, ME). Animals were group-housed two to three per cage in a temperature- and humidity-controlled, pathogen-free vivarium, with ad libitum access to food and water. Lighting was on a 12 h/12 h cycle, with lights on from 06:00 h. Body weights were measured during the month prior to testing. The NIMH Intramural Research Program Animal Care and Use Committee approved all procedures, which conformed to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Health screen and sensorimotor function tests

Mice were first evaluated for appearance, weight gain, general activity, and behavior in their home cages, as described by Crawley and coworkers [12,13,37]. A checklist modified from that of Paylor et al. [38] was designed to record results of challenges intended to detect any gross abnormalities and to evaluate reflex responses and sensorimotor abilities. Eye-blink, ear-twitch, whisker-touch, righting, and postural reflexes were evaluated. Each mouse was observed for 3 min in a clean, empty cage for condition of fur, presence of whiskers, freezing behavior, defecation, and urination [27]. Olfactory acuity (latency to sniff/locate buried peanut butter) was also measured.

Balance and grip strength were evaluated in simple tests of motor responses. In a modification from Paylor et al. [38], each mouse was suspended over a litter-filled cage bottom from a standard wire cage lid. Edges were taped to prevent escape to the opposite or "top" side. The number of seconds each mouse was able to hang onto the wire lid without falling was recorded, to a maximum of 60 s. For the pole climbing test, each mouse was placed in the center of a 1.5-cm diameter wooden dowel covered with cloth tape. One end of the dowel was slowly lifted to a vertical position so that the mouse was facing up. The time each mouse was able

to hang onto or climb the pole was recorded, to a maximum of 60 s.

2.3. Circadian wheel running

WT ($n = 8$) and p50 KO ($n = 8$) mice were placed in an isolated room on a 12 h/12 h light/dark cycle. Each animal was individually housed in a Naglene cage with a running wheel. Revolutions were tracked with VitalView software (apparatus and software from Mini-Mitter Co., Sun River, OR), and food and water were available ad libitum. Running wheel data (actograms) were collected continuously over 5 days. Data from the last 3 days of recording were averaged for final determination of total revolutions/min/period and relative activity in the light versus dark phases.

2.4. Open field exploration

After being acclimated to the procedure room for at least 30 min, each of 20 WT and 19 p50 KO mice was individually videotaped during a 5-min exploration session in a 40 cm × 40 cm Plexiglas Digiscan open field activity box (Accuscan, Columbus, OH) containing horizontal infrared photocell sensors. Mice were placed in the center of the arena at the beginning of the test period. Their movement around the arena was recorded by a portable video camera vertically mounted on a tripod 3 ft above and controlled remotely by the experimenter in the same room. The Plexiglas box was cleaned after each individual test session to prevent subsequent mice from being influenced by odors deposited by previous mice. Testing was performed under normal fluorescent room lights (800 lx), during the light phase of the circadian cycle, between 09:30 and 15:30 h. Locomotor activity was recorded by the photocell analyzer as the number of horizontal beam breaks and total number of movements. The number of fecal boli deposited, time spent in the center of the box, and time spent along the perimeter of the box were analyzed as a measure of anxiety-like behavior [14] and manually scored while reviewing the videotapes. The center comprised the central 4 squares of the field divided into 16 equal-sized squares.

2.5. Elevated plus maze

Testing was conducted in the same procedure room with a different set of mice under the same conditions as the Open field test, between 09:30 and 15:30 h. The maze consisted of four 5 cm × 29 cm orthogonal arms connected by a 5 cm × 5 cm central square. Two arms of the maze, on opposite sides, were enclosed by non-transparent sidewalls, and the other two arms were open with no lip. The apparatus was elevated 40 cm above the floor. Following brief adaptation to the room, a mouse was individually removed from its home cage and placed on the center of the maze, facing an open arm. The behavior of each mouse was recorded by a video camera mounted directly above the maze. During the 5-min test period, conventional parameters of anxiety-like behavior were monitored, i.e., the number of entries into the closed arms, entries into the open arms, and the total time spent in each arm. Scoring was done manually while reviewing the videotapes. Entry into an arm of the maze was scored if the center of the mouse's body crossed the opening into that arm.

2.6. Habituation and novel object test

To examine how the mice adapt to an environment and respond to a novel object [19], the animals were placed in the open field box with a central divider (20 cm × 40 cm). Mice were left undisturbed in the box for 1 h on each of 3 days. The room was quiet and brightly lit; behavior was recorded by video camera and analyzed by EthoVision 2.3 software (Noldus, Wageningen, The Netherlands). Activity (distance traveled) and position (location in rectangle) were recorded. On the third day, at 30 min, a novel object (jar lid, 5.5 cm diameter × 1 cm high, covered with light-blue labeling tape) was placed in one end of the rectangular field, approximately 10 cm from the edges. Time spent in the 20 cm × 20 cm side of the box with the novel object relative to the non-object 20 cm × 20 cm side was recorded for the remaining 30 min of the test session. The test is designed to measure the tendency of an animal to explore a novel object in a situation of minimally perceived threat, to better assess the strength of one of the two competing drives (exploration versus safety) [10].

2.7. Statistical analyses

Data are expressed as mean ± S.E.M. and were compared by non-parametric (Mann–Whitney U or Chi square) tests for choice and event occurrence data and parametric T tests for temporal and activity data. Significance was set to $P < 0.05$.

3. Results

3.1. Preliminary observations

All mice displayed good general health. Home-cage behaviors, including sleeping patterns, group huddling, and general activity, appeared normal for both p50 KO and WT mice. There were no differences noted in sniffing, rearing, urination, righting reflexes, eye-blink, whisker and ear-twitch responses, or balance maintenance. However, body weight, fur allogrooming, whisker barbering, self-grooming behavior, and defecation differed significantly between the genotypes (Table 1).

Relative to WT, p50 KO mice weighed less at every time point after arrival in the animal facility at 2 months of age. Although the weight differences were small (9%), they were significant at 3 months of age ($P < 0.002$). Bald patches

Table 1
Sensorimotor function assessed in WT and p50 KO mice

Behavior	Wild type ($n = 29$)	p50 KO ($n = 29$)	Significance
Physical characteristics			
Weight (g)	29 ± 1	26 ± 1	$P < 0.002$
Whisker barbering (% showing)	69	10	$P < 0.0001$
Bald patches (% showing)	31	7	$P < 0.05$
Behavior in test environment[a]			
Freezing (% showing)	41	0	$P < 0.005$
Grooming (% showing)	93	72	$P < 0.05$
Jumping (% showing)	0	17	$P < 0.05$
Fecal boli/mouse	2.0 ± 0.3	0.5 ± 0.3	$P < 0.0001$
Urination (% showing)	38	28	NS
Motor responses (seconds to fall; 60 s maximum)			
Wire suspension	47 ± 3	53 ± 3	NS
Pole test	41 ± 4	25 ± 4	$P < 0.01$
Sensorimotor reflexes[b]			
Olfactory (latency to sniff; s)	15 ± 2	16 ± 2	NS

Sum of three sets of mice, all tested about the same age (12 weeks). Measures are instances per group or means ± S.E.M. in a 3-min test session. Non-parametric statistics were used.

[a] Normal: palpebral closure, exophthalmous (none), piloerection (none), sniffing, licking, grooming, rearing, moving throughout cage.

[b] Normal: righting, postural, whisker reflexes, eye-blink, pupil constriction, ear-twitch, maintain balance.

were present in 7% of the p50 KO mice versus 31% of WT mice ($P < 0.05$). Whereas only 10% of p50 KO mice had whiskers that were barbered, 69% of the WT mice had short or missing whiskers ($P < 0.0001$). The monitoring of anxiety-like behavior during each mouse's 3-min exploration of a clean, empty cage revealed more incidences of freezing and defecation by WT relative to KO mice (Table 1). Interestingly, 41% of WT mice displayed freezing behavior while being observed, but no KO mice did so ($P < 0.005$).

When challenged with wire hanging and pole climbing tasks as informal measures of strength [12], p50 KO mice did not differ from WT mice in latency to fall from an inverted wire cage lid in a 60-s test (Table 1). However, WT mice hung onto the tilted pole 64% longer than p50 KO mice ($P < 0.01$).

3.2. Circadian wheel running

Over the final three of five days housed in the wheel running cages, both genotypes showed equivalent revolutions/min/period of wheel running, In terms of total activity (WT: 2.90±0.57; KO: 3.78±0.9), activity in the light phase (WT: 0.23 ± 0.10; KO: 0.30 ± 0.07), activity in the dark phase (WT: 5.60 ± 1.16; KO: 7.26 ± 1.86), and light:dark ratio (WT: 0.065 ± 0.034; KO: 0.068 ± 0.024), none of the differences was statistically significant. Fig. 1 shows representative actograms from individual animals of each genotype.

3.3. Open field exploration

Locomotor activity and anxiety-like responses of p50 KO and WT mice were measured in a novel open field. As shown in Fig. 2, p50 KO mice spent almost twice as much time as the WT mice ($P < 0.001$) in the center square, an anxiogenic location. Locomotor activity also differed between the genotypes, with 50% more total ambulation displayed by p50 KO mice ($P < 0.001$). p50 KO mice showed higher numbers of total rearing in the open field, and only the KO mice reared in the center square (data not shown).

3.4. Elevated plus maze

When challenged in the elevated plus maze, all mice demonstrated preference for closed arms, as open arms classically represent an anxiogenic situation for rodents [39]. However, p50 KO mice spent 3.6-fold more time on the open arms of the maze relative to WT ($P < 0.01$), and the percent time spent in the open arm was also significantly greater ($P < 0.05$) (Fig. 2). Overall, KO mice were more active on the maze and made about 50% more total arm entries than the WT mice ($P < 0.05$) (Fig. 2).

3.5. Habituation and novel object tests

In the habituation/novelty test, on day 1, the KO mice were initially more active than WT mice in the rectangular open field, with 56% more cumulative activity over the session ($P < 0.05$) (Fig. 3a), but they showed similar levels of activity on days 2 and 3 relative to WT mice (differences non-significant) (Fig. 3b and c). Over the course of the 3 days, WT mice decreased their activity (in the first half of the session) to 62% and KO mice to 55% of starting levels ($P < 0.05$ for both groups). Upon presentation of the novel object in one end of the open field, both groups showed small increases in activity, with the KO mice showing a greater but

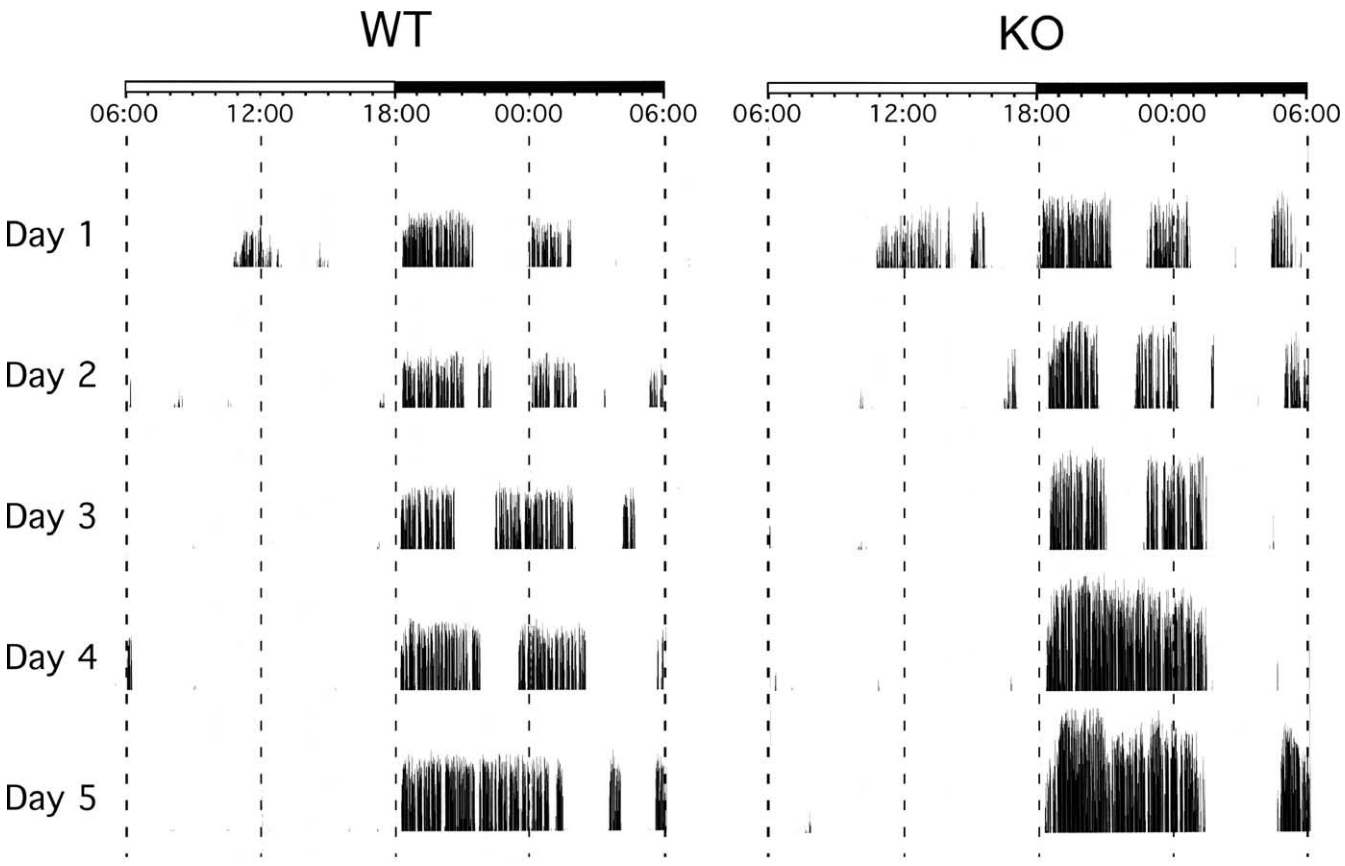

Fig. 1. Two representative wheel running actograms from individual WT and p50 KO mice are shown. When mice are individually placed in cages in a strictly controlled 12 h/12 h light/dark cycle with access to running wheels, they quickly entrain to the photoperiod and are active mainly in the dark. No differences were noted between genotypes (see text for details).

non-significant increase relative to WT (Fig. 3c). WT mice avoided the side containing the novel object ($P < 0.05$), whereas KO mice did not show a side preference and spent significantly more time in proximity to the novel object than did the WT mice ($P < 0.01$) (Fig. 3d).

4. Discussion

The present study examined the contribution of the p50 subunit of the NF-κB transcription factor to emotional behavior. Ubiquitous deletion of the *NFKB1* gene in all cells results in deletion of p50 protein and altered NF-κB function at the cellular and organ levels. The p50 KO mouse develops normally, shows selective defects in immune responses [45,48], and now has been shown to have a distinctive behavioral phenotype characterized by decreased anxiety-like responses, elevated exploratory behavior, and reduced tendency to establish dominant–subordinate relationships amongst cage mates.

Several formal tests of anxiety-like behavior were conducted. Because mice have a natural aversion to the brightly lit center of an open field, comparison of activity in the center versus the periphery is an indication of anxiety-related behavior [12]. Similarly, in the elevated plus maze, exploration of an open arm is in conflict with aversion to open areas and height. Finally in the novel object test in a familiar environment, the natural desire to explore the object conflicts with perceived danger associated with the same object. In all these tests, the p50 mutant mice displayed a behavioral profile that was different from the WT mice. Whereas the parent B6129 strain showed typical behaviors reflecting a natural caution posed by ethological challenges, the p50 KO mouse failed to show these behaviors and instead was more likely to explore the open center of the field and enter the open arms of the elevated plus maze more often (Fig. 2). In the habituation/novelty test, the p50 KO mouse showed less avoidance behavior than the WT mouse upon presentation of a novel object (Fig. 3).

Naturalistic observations bear out the striking phenotype of the p50 KO mouse. In a novel environment, these mice showed significantly less defecation and freezing behavior (Table 1). The KO mice were normal in all tests of sensorimotor function except for the pole test, in which they were more inclined than the WT to jump off while it was being elevated. This tendency is more likely to be related to their reduced anxiety rather than muscle weakness, because the p50 KO mice were normal in the wire suspension task.

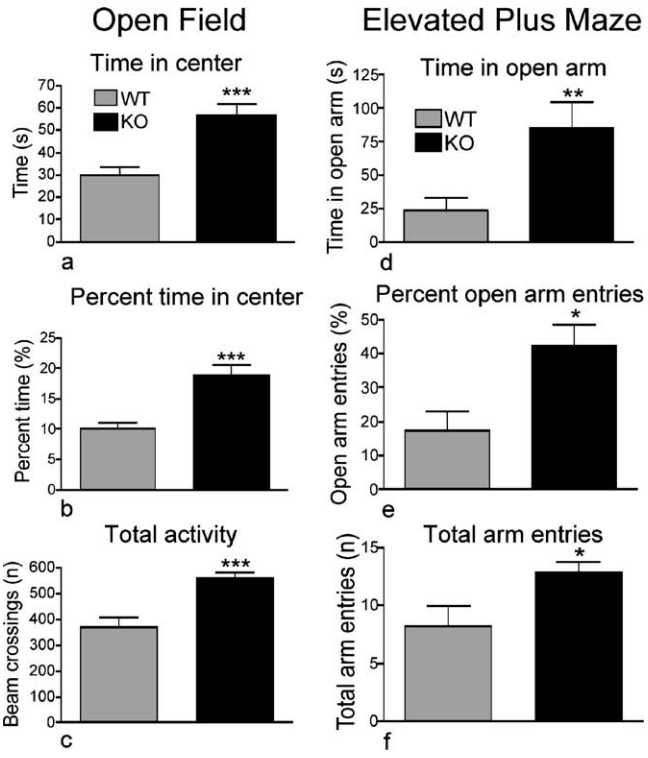

Fig. 2. Behavior in the open field (a–c) and elevated plus maze (d–f) is shown for p50 KO and WT mice. In the 5-min test period in the open field, KO mice ($n = 19$) and WT ($n = 20$) were compared for time spent in center (a), percent of total time spent in center (b), and total activity in the field (c). In the elevated plus maze, different groups of p50 KO ($n = 9$) and WT ($n = 12$) were analyzed by videotape analysis for time spent in open arm (d), percent of open arm entries as a function of total arm entries (e), and total arm entries (f). This experiment was repeated once with similar results. *T* tests were used on time measures, and Mann–Whitney *U* tests were used on entry measures. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. WT mice.

Missing or shortened whiskers and bald patches on face and upper body (alopecia) created by whisker and fur chewing (pulling) occurs when social hierarchies are established, allowing cooperative dominant and subordinate behaviors amongst cage mates [42]. This process, which has a genetic component, occurs less in the p50 KO mice relative to WT mice, suggesting that normal hierarchical social interaction is deficient in the mutant.

In all presentations of a novel environment in this study, the p50 KO mice were initially more active. However, these mice are not naturally hyperactive; they showed normal behavior and circadian rhythmicity in the running wheels. They also adapted to an open field by the second day of the habituation test, thereafter showing normal activity levels relative to WT mice until a novel object was placed in the field; the KO mice avoided the side containing the object less than the WT mice did, suggesting that reduced anxiety more likely accounts for the difference rather than increased novelty seeking or exploratory behavior.

KO mice are normal in simple sensorimotor assessments, and they perform similar to WT in tests of cognitive function. In a previous study, it was concluded that p50 KO mice exhibited selective memory deficits, based on their reduced tendency to avoid footshock by learning the location of an escape platform [22]. It is possible that p50 KO mice do not perceive anxiety-evoking stimuli in the same way as WT mice. Such “lack of appreciation” of a fear-inducing stimulus may account for the performance differences in the KO mice that were interpreted as an inability to learn or remember. Further tests of cognitive function utilizing both appetitive and aversive tasks are warranted.

The mechanistic bases of the behavioral phenotype of the p50 KO mouse are unknown and remain to be explored in greater detail. The genetic deletion in these mice affects cellular processes throughout the body. One possible explanation for the relative lack of anxiety and natural fear responses is that a deficit in the peripheral immune system is rendering the animals chronically sick in a manner that might affect performance in anxiety tests. In fact, p50 KO mice are prone to infections and tend to live shorter lifespans than WT mice [4,45], but they have normal innate immune responses and normal levels of circulating cytokines [4,24] that might be a causal factor in affecting CNS function [25]. Further studies are needed to assess these parameters. However, the sickness hypothesis predicts that these animals would show reduced activity, and this is not the case. p50 KO mice appear to be normal, alert, and healthy by routine examination. They also appear to gain weight at similar rates over the course of the several months they are in the facility.

Alternatively, brain circuits subserving anxiety- and/or fear-like behaviors may be modified by the defective NF-κB signaling in cells of the p50 KO mice. It is not known whether defective signaling as a result of p50 deletion occurs to a significant degree in emotional circuits in the brain or even whether neurons or glia are contributing to the phenotypic alterations. A striking study by Yeh et al. does support a crucial role for NF-κB in emotional learning. In that study, specific NF-κB transcription factor activity in the amygdala was shown to be required for fear conditioning [51].

Little is known about the role of NF-κB in normal brain function, thus only a few speculative comments can be made. Although there are reports arguing for a role for NF-κB in normal neuronal development [11] (but possibly not for the p105/p50 protein [43]), to our knowledge there are no reports of neuropathology in the p50 KO mouse. In the absence of prototypical p50/p65 heterodimers, other combinations of Rel proteins may adequately compensate for the deletion, allowing for the development of normal brain anatomy. Because p50 homodimers have been shown to repress transcription [44], absence of p50 may result in elevated NF-κB-mediated transcriptional activity [23].

Cultured hippocampal neurons from p50 KO mice show enhanced intracellular Ca^{2+} levels following glutamate exposure [52]. Yeh et al. [51] showed that CREB and NF-κB (marked by a p50 antibody) interact in the cell nucleus. It

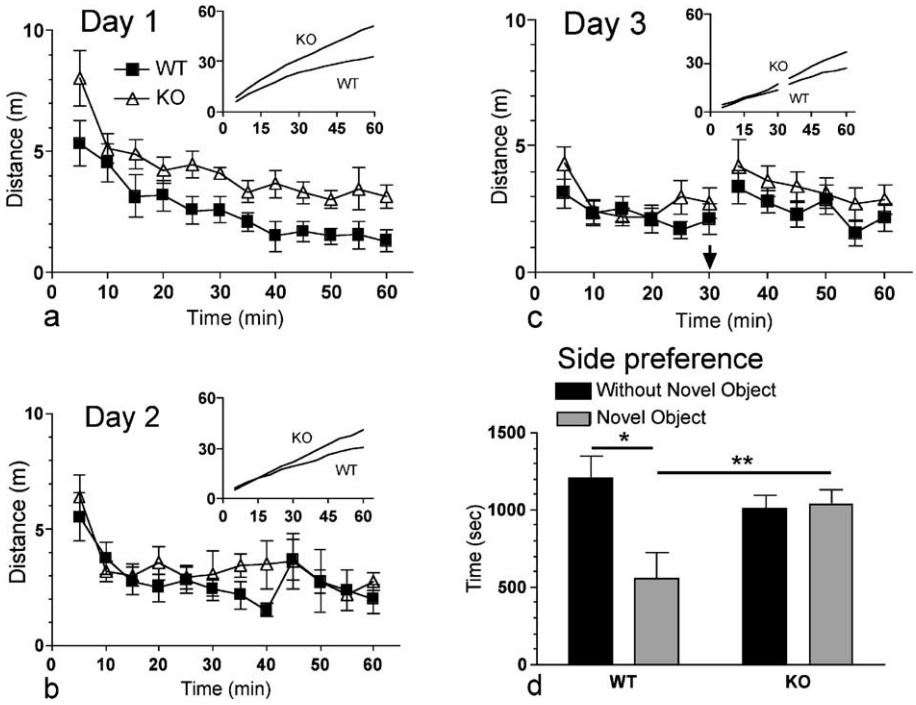

Fig. 3. Habituation of activity in a rectangular 20 cm × 40 cm open field and time spent in the side containing a novel object were measured by automated tracking software for WT ($n = 10$) and p50 KO ($n = 9$) mice across 3 days of testing. Data are presented as means ± S.E.M. of distance traveled per 5-min time bin or cumulatively across the session (insets). KO mice showed significantly more cumulative activity than WT mice on day 1 (a) during a 1-h test session ($P = 0.013$) but showed similar activity levels on days 2 (b) and 3 (c). Upon presentation of a novel object (taped lid) in one end of the field at 30 min of day 3, KO mice spent significantly more time on the side containing the object than did WT mice (d). Analyses by T test: $^{*}P < 0.05$, $^{**}P < 0.01$.

is thus possible that cells lacking p50 have elevated Ca^{2+} levels and altered NF-κB/CREB interactions upon synaptic stimulation. Altered CREB activity in the striatum has been implicated in gating affective behavior [7]. It is also noteworthy that ΔFosB overexpression strongly upregulates striatal *NFKB1* gene expression [3], suggesting several forms of interaction between these transcription factors. Future studies are needed to show how the interactions affect output measures in vitro and in vivo.

The present study links altered NF-κB function with reduced anxiety-like behavior in several non-learning paradigms exploiting naturalistic ethological conflicts. A genetic basis for emotional states is well known, and there are multiple candidate genes based on animal models [15]. Few of the candidate genes are immune genes, however. Altered cytokine levels or cytokine gene loci have been correlated with anxiety-like behaviors in some studies [26,33,36,50]. The present study is the first to implicate involvement of the key transcription factor of the immune system, NF-κB, in manifestation of affective behavior.

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References

[1] Albensi BC, Mattson MP. Evidence for the involvement of TNF and NF-κB in hippocampal synaptic plasticity. Synapse 2000;35:151–9.
[2] Alcamo E, Mizgerd JP, Horwitz BH, Bronson R, Beg AA, Scott M, et al. Targeted mutation of TNF receptor I rescues the RelA-deficient mouse and reveals a critical role for NF-κB in leukocyte recruitment. J Immunol 2001;167:1592–600.
[3] Ang E, Chen J, Zagouras P, Magna H, Holland J, Schaeffer E, et al. Induction of nuclear factor-κB in nucleus accumbens by chronic cocaine administration. J Neurochem 2001;79:221–4.
[4] Artis D, Speirs K, Joyce K, Goldschmidt M, Caamano J, Hunter CA, et al. NF-κB1 is required for optimal $CD4^{+}$ Th1 cell development and resistance to *Leishmania major*. J Immunol 2003;170:1995–2003.
[5] Bakalkin G, Yakovleva T, Terenius L. NF-κB-like factors in the murine brain. Developmentally-regulated and tissue-specific expression. Brain Res Mol Brain Res 1993;20:137–46.
[6] Barkett M, Gilmore TD. Control of apoptosis by Rel/NF-κB transcription factors. Oncogene 1999;18:6910–24.
[7] Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, et al. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. Proc Natl Acad Sci USA 2002;99:11435–40.
[8] Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, et al. Control of synaptic strength by glial TNFα. Science 2002;295:2282–5.
[9] Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-κB. Nature 1995;376:167–70.
[10] Besheer J, Bevins RA. The role of environmental familiarization in novel-object preference. Behav Process 2000;50:19–29.

[11] Bhakar AL, Tannis LL, Zeindler C, Russo MP, Jobin C, Park DS, et al. Constitutive nuclear factor-κB activity is required for central neuron survival. J Neurosci 2002;22:8466–75.
[12] Crawley J. What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. New York: Wiley-Liss; 2000. 329 pp.
[13] Crawley JN, Belknap JK, Collins A, Crabbe JC, Frankel W, Henderson N, et al. Behavioral phenotypes of inbred mouse strains: implications and recommendations for molecular studies. Psychopharmacology (Berl) 1997;132:107–24.
[14] DeFries JC, Hegmann JP, Halcomb RA. Response to 20 generations of selection for open-field activity in mice. Behav Biol 1974;11:481–95.
[15] Finn DA, Rutledge-Gorman MT, Crabbe JC. Genetic animal models of anxiety. Neurogenetics 2003;4:109–35.
[16] Freudenthal R, Romano A. Participation of Rel/NF-κB transcription factors in long-term memory in the crab *Chasmagnathus*. Brain Res 2000;855:274–81.
[17] Fridmacher V, Kaltschmidt B, Goudeau B, Ndiaye D, Rossi FM, Pfeiffer J, et al. Forebrain-specific neuronal inhibition of nuclear factor-κB activity leads to loss of neuroprotection. J Neurosci 2003;23:9403–8.
[18] Gratacos M, Nadal M, Martin-Santos R, Pujana MA, Gago J, Peral B, et al. A polymorphic genomic duplication on human chromosome 15 is a susceptibility factor for panic and phobic disorders. Cell 2001;106:367–79.
[19] Hashimoto H, Shintani N, Tanaka K, Mori W, Hirose M, Matsuda T, et al. Altered psychomotor behaviors in mice lacking pituitary adenylate cyclase-activating polypeptide (PACAP). Proc Natl Acad Sci USA 2001;98:13355–60.
[20] Kaltschmidt C, Kaltschmidt B, Baeuerle PA. Stimulation of ionotropic glutamate receptors activates transcription factor NF-κB in primary neurons. Proc Natl Acad Sci USA 1995;92:9618–22.
[21] Kaltschmidt C, Kaltschmidt B, Neumann H, Wekerle H, Baeuerle PA. Constitutive NF-κB activity in neurons. Mol Cell Biol 1994; 14:3981–92.
[22] Kassed CA, Willing AE, Garbuzova-Davis S, Sanberg PR, Pennypacker KR. Lack of NF-κB p50 exacerbates degeneration of hippocampal neurons after chemical exposure and impairs learning. Exp Neurol 2002;176:277–88.
[23] Kastenbauer S, Ziegler-Heitbrock HW. NF-κB1 (p50) is upregulated in lipopolysaccharide tolerance and can block tumor necrosis factor gene expression. Infect Immun 1999;67:1553–9.
[24] Kato A, Edwards MJ, Lentsch AB. Gene deletion of NF-κB p50 does not alter the hepatic inflammatory response to ischemia/reperfusion. J Hepatol 2002;37:48–55.
[25] Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 2002; 25:154–9.
[26] Kustova Y, Sei Y, Morse Jr HC, Basile AS. The influence of a targeted deletion of the IFNγ gene on emotional behaviors. Brain Behav Immun 1998;12:308–24.
[27] LeDoux JE. Emotion: clues from the brain. Annu Rev Psychol 1995;46:209–35.
[28] Li Q, Verma IM. NF-κB regulation in the immune system. Nat Rev Immunol 2002;2:725–34.
[29] Liou HC, Baltimore D. Regulation of the NF-κB/rel transcription factor and IκB inhibitor system. Curr Opin Cell Biol 1993;5:477–87.
[30] Maes M. Evidence for an immune response in major depression: a review and hypothesis. Prog Neuropsychopharmacol Biol Psychiatry 1995;19:11–38.
[31] Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D. NF-κB functions in synaptic signaling and behavior. Nat Neurosci 2003;6:1072–8.
[32] Middle F, Jones I, Robertson E, Lendon C, Craddock N. Tumour necrosis factor alpha and bipolar affective puerperal psychosis. Psychiatr Genet 2000;10:195–8.
[33] Nakamura K, Xiu Y, Ohtsuji M, Sugita G, Abe M, Ohtsuji N, et al. Genetic dissection of anxiety in autoimmune disease. Hum Mol Genet 2003;12:1079–86.
[34] Ninan PT. Dissolving the burden of generalized anxiety disorder. J Clin Psychiatry 2001;62(Suppl 19):5–10.
[35] Park HJ, Lee HS, Lee HJ, Yoo YM, Kim SA, Leem K, et al. Decrease of the electroacupuncture-induced analgesic effects in nuclear factor-kappa B1 knockout mice. Neurosci Lett 2002;319:141–4.
[36] Pawlak CR, Ho YJ, Schwarting RK, Bauhofer A. Relationship between striatal levels of interleukin-2 mRNA and plus-maze behaviour in the rat. Neurosci Lett 2003;341:205–8.
[37] Paylor R, Hirotsune S, Gambello MJ, Yuva-Paylor L, Crawley JN, Wynshaw-Boris A. Impaired learning and motor behavior in heterozygous *Pafah1b1* (*Lis1*) mutant mice. Learn Mem 1999;6:521–37.
[38] Paylor R, Nguyen M, Crawley JN, Patrick J, Beaudet A, Orr-Urtreger A. α7 nicotinic receptor subunits are not necessary for hippocampal-dependent learning or sensorimotor gating: a behavioral characterization of Acra7-deficient mice. Learn Mem 1998;5:302–16.
[39] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
[40] Pugh CR, Nguyen KT, Gonyea JL, Fleshner M, Wakins LR, Maier SF, et al. Role of interleukin-1 beta in impairment of contextual fear conditioning caused by social isolation. Behav Brain Res 1999;106:109–18.
[41] Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry 2003;160:1554–65.
[42] Sarna JR, Dyck RH, Whishaw IQ. The Dalila effect: C57BL6 mice barber whiskers by plucking. Behav Brain Res 2000;108:39–45.
[43] Schmidt-Ullrich R, Memet S, Lilienbaum A, Feuillard J, Raphael M, Israel A. NF-κB activity in transgenic mice: developmental regulation and tissue specificity. Development 1996;122:2117–28.
[44] Schmitz ML, Baeuerle PA. The p65 subunit is responsible for the strong transcription activating potential of NF-κB. EMBO J 1991;10:3805–17.
[45] Sha WC, Liou HC, Tuomanen EI, Baltimore D. Targeted disruption of the p50 subunit of NF-κB leads to multifocal defects in immune responses. Cell 1995;80:321–30.
[46] Tarantino LM, Bucan M. Dissection of behavior and psychiatric disorders using the mouse as a model. Hum Mol Genet 2000;9:953–65.
[47] Villafuerte S, Burmeister M. Untangling genetic networks of panic, phobia, fear and anxiety. Genome Biol 2003;4:224.
[48] Weih F, Durham SK, Barton DS, Sha WC, Baltimore D, Bravo R. p50-NF-κB complexes partially compensate for the absence of RelB: severely increased pathology in $p50^{-/-}relB^{-/-}$ double-knockout mice. J Exp Med 1997;185:1359–70.
[49] Weiss SM, Lightowler S, Stanhope KJ, Kennett GA, Dourish CT. Measurement of anxiety in transgenic mice. Rev Neurosci 2000;11:59–74.
[50] Yamada K, Iida R, Miyamoto Y, Saito K, Sekikawa K, Seishima M, et al. Neurobehavioral alterations in mice with a targeted deletion of the tumor necrosis factor-α gene: implications for emotional behavior. J Neuroimmunol 2000;111:131–8.
[51] Yeh SH, Lin CH, Lee CF, Gean PW. A requirement of nuclear factor-κB activation in fear-potentiated startle. J Biol Chem 2002;277:46720–9.
[52] Yu Z, Zhou D, Bruce-Keller AJ, Kindy MS, Mattson MP. Lack of the p50 subunit of nuclear factor-κB increases the vulnerability of hippocampal neurons to excitotoxic injury. J Neurosci 1999;19:8856–65.
[53] Yu Z, Zhou D, Cheng G, Mattson MP. Neuroprotective role for the p50 subunit of NF-κB in an experimental model of Huntington's disease. J Mol Neurosci 2000;15:31–44.