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Reduction of Endogenous Kynurenic Acid Formation Enhances Extracellular Glutamate, Hippocampal Plasticity, and Cognitive Behavior

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At endogenous brain concentrations, the astrocyte-derived metabolite kynurenic acid (KYNA) antagonizes the α7 nicotinic acetylcholine receptor and, possibly, the glycine co-agonist site of the NMDA receptor. The functions of these two receptors, which are intimately involved in synaptic plasticity and cognitive processes, may, therefore, be enhanced by reductions in brain KYNA levels. This concept was tested in mice with a targeted deletion of kynurenine aminotransferase II (KAT II), a major biosynthetic enzyme of brain KYNA. At 21 days of age, KAT II knock-out mice had reduced hippocampal KYNA levels (−71%) and showed significantly increased performance in three cognitive paradigms that rely in part on the integrity of hippocampal function, namely object exploration and recognition, passive avoidance, and spatial discrimination. Moreover, compared with wild-type controls, hippocampal slices from KAT II-deficient mice showed a significant increase in the amplitude of long-term potentiation *in vitro*. These functional changes were accompanied by reduced extracellular KYNA (−66%) and increased extracellular glutamate (+51%) concentrations, measured by hippocampal microdialysis *in vivo*. Taken together, a picture emerges in which a reduction in the astrocytic formation of KYNA increases glutamatergic tone in the hippocampus and enhances cognitive abilities and synaptic plasticity. Our studies raise the prospect that interventions aimed specifically at reducing KYNA formation in the brain may constitute a promising molecular strategy for cognitive improvement in health and disease. *Neuropsychopharmacology* (2010) **35,** 1734–1742; doi:10.1038/npp.2010.39; published online 24 March 2010

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INTRODUCTION

Kynurenic acid (KYNA), a neuroinhibitory brain metabolite (Perkins and Stone, 1982), is produced by the irreversible transamination of kynurenine in a side arm of the kynurenine pathway of tryptophan degradation. In the mammalian brain, KYNA is synthesized by distinct kynurenine aminotransferases (KATs), of which KAT II has a major function (Guidetti *et al*, 2007a; Han *et al*, 2009). KAT II is localized almost exclusively in astrocytes (Guidetti *et al*, 2007b), which rapidly release newly formed KYNA into the extracellular milieu (Kiss *et al*, 2003). By competing with the endogenous agonists glycine and D-serine, KYNA may then act as an antagonist of the glycine co-agonist site of the NMDA receptor (NMDAR; Kessler *et al*, 1989). More likely, however, endogenous KYNA functions as an antagonist of the allosteric-potentiating ligand site of the α7 nicotinic acetylcholine receptor (α7nAChR) (Lopes *et al*, 2007). Alone or jointly, these receptors account for KYNA's ability to reduce brain excitation and are responsible for its neuroprotective and anticonvulsive properties (Foster *et al*, 1984). These effects may have a pathophysiologically relevant function in neurodegenerative diseases such as Huntington disease and Alzheimer disease, which present with abnormal brain KYNA levels (see Vámos *et al*, 2009 for review).

By virtue of its ability to inhibit the NMDAR and the α7nAChR, endogenous KYNA might also be involved in cognitive functions. Both these receptors are critical for physiological processes underlying learning, memory and, more generally, synaptic plasticity (MacDonald *et al*, 2006; Albuquerque *et al*, 2009). Therefore, fluctuations in KYNA levels can be envisioned to influence these and related phenomena. This concept is supported by studies showing that increases in brain KYNA levels, caused by the administration of kynurenine or by shifting kynurenine pathway metabolism toward enhanced KYNA formation, result in cognitive impairments in experimental animals.

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For example, elevated KYNA levels disrupt prepulse inhibition and auditory sensory gating (Shepard *et al*, 2003; Erhardt *et al*, 2004) and induce deficits in contextual learning and memory (Chess and Bucci, 2006; Chess *et al*, 2007). These deleterious effects of KYNA model aspects of cognitive deficits observed in schizophrenia and are, therefore, especially interesting in view of the fact that schizophrenia patients present with increased KYNA levels in brain and cerebrospinal fluid (Erhardt *et al*, 2001; Schwarcz *et al*, 2001).

This study was designed to test the complementary hypothesis, namely that a selective *reduction* in endogenous KYNA levels will, conversely, *improve* cognitive abilities. To this end, we generated mice with a targeted deletion of KAT II (KAT II knock-out (KO) mice; FVB/N background) and compared biochemical, behavioral, and electrophysiological characteristics of these mutant animals with those of wild-type (WT) controls. The studies described here were conducted in, or are related to, the hippocampus, a brain area central to cognitive processing (Morris, 2006) and abundantly endowed with both NMDARs and α7nAChRs (Monaghan *et al*, 1989; Fabian-Fine *et al*, 2001). Using hippocampal *in vivo* microdialysis, we show that KAT II KO mice show the expected decrease in extracellular KYNA levels, and that this reduction is accompanied by a significant increase in extracellular glutamate. We propose that these chemical changes may be causally related to the fact that KAT II-deficient animals show improved performance in three cognition paradigms and enhanced long-term potentiation (LTP) in hippocampal tissue slices.

MATERIALS AND METHODS

Animals

Twenty-one-day-old WT and KAT II KO mice were used in all studies. To optimize the behavioral readout (Brooks *et al*, 2005; Pollak *et al*, 2005), mutant mice were generated by backcrossing homozygous 129/SvEv KAT II KO mice (Yu *et al*, 2004) with WT FVB/N mice (Supplementary Materials and Methods; Supplementary Figure S1). Animals were housed in temperature-controlled animal facilities on a 12h/12 h light dark cycle with free access to food and water. The facilities were fully accredited by the American Association for the Accreditation of Laboratory Animal Care and the Canadian Council of Animal Care, respectively, and the experimental protocols followed the 'Principles of Laboratory Animal Care' (NIH publication No. 86–23, 1996).

Chemicals

KYNA, glutamate, and other fine biochemicals were purchased from Sigma Chemical (St Louis, MO). AP-5, NBQX, bicuculline, picrotoxin, and strychnine were obtained from RBI (Natick, MA). CGP-52532 was purchased from Tocris (Bristol, UK). All other chemicals were obtained from a variety of suppliers and were of the highest commercially available purity.

Microdialysis

Mice were anesthetized with chloral hydrate (360 mg/kg, i.p.) and mounted in a David Kopf stereotaxic frame. A guide cannula (outer diameter: 0.65 mm) was implanted unilaterally over the dorsal hippocampus (AP: 1.6 mm posterior to bregma, L: 1.1 mm from midline, V: 1.1 mm below the skull surface) and secured to the skull with an anchor screw and acrylic dental cement. On the next day, a microdialysis probe (CMA10, Carnegie Medicin, Stockholm, Sweden; membrane length: 1 mm) was inserted through the guide cannula and connected to a microinfusion pump set to a speed of 1 μl/min and then perfused with Ringer solution containing (in mM) NaCl, 144; KCl, 4.8; $MgSO_4$, 1.2; $CaCl_2$, 1.7; pH 6.7. Samples were collected every 30 min for the duration of the experiment.

Chemical Analyses

KYNA determination in tissue. Animals were euthanized (CO_2), and their hippocampi were dissected out without delay and sonicated in ultrapure water (1 : 10, w/v). A total of 100 μl of the homogenate were acidified with 25 μl of 6% perchloric acid. After centrifugation (10 min, 12 000 *g*), 20 μl of the supernatant were subjected to HPLC analysis, and KYNA was detected fluorimetrically (cf. Supplementary Materials and Methods). Data were analyzed using an unpaired Student's *t*-test.

KYNA and glutamate determination in microdialysate. To determine the KYNA content of microdialysis samples, 15 μl of the perfusate were injected directly into the same HPLC system used for tissue analysis (see above).

The glutamate content of microdialysis samples (15 μl) was determined by HPLC analysis with fluorimetric detection, as reported by Shank *et al* (1993) (cf. Supplementary Materials and Methods).

Microdialysis data were analyzed by two-way repeated measures ANOVA (genotype × time) followed by Bonferroni's *post hoc* test for multiple comparisons.

Behavioral Analyses

Object exploration and recognition test. The object exploration and recognition task (adapted from Save *et al*, 1992) analyzed four different parameters: locomotor activity (open field), object exploration (habituation to environment), object displacement (response to spatial change), and object recognition (response to a novel object, NO) using the arena and video recording device described in Supplementary Materials and Methods. The procedure involved seven consecutive 6-min sessions with ~30 s inter-session intervals. All sessions were scored from the video film. Object exploration was characterized by the amount of time the subject spent in contact with, or sniffing, an object.

Data analysis was conducted as follows: genotype differences in habituation (S2–S4) were analyzed using a two-way repeated measures ANOVA (genotype × session). The capacity to recognize displaced objects (DOs) in the environment was analyzed by comparing exploration of the DOs (minus the respective baseline value for each genotype)

using a two-way repeated measures ANOVA (genotype × session). *Post hoc* analysis was conducted using univariate contrast analysis. NO recognition was analyzed by one-way ANOVA (genotype), using the ratio between the time spent exploring the NO *vs* the average time spent on all other objects (both DOs and non-DOs).

Contextual memory. The passive avoidance apparatus had two compartments of equal size, one illuminated and the other in darkness, separated by a guillotine door (Supplementary Materials and Methods). On day 1, the animal was first placed in the illuminated compartment. The door was then opened, prompting the mouse to move rapidly into the preferred dark compartment. After the door was closed, an inescapable foot shock (0.56 mA for 1 s) was delivered through metal rods of the floor. The latency to enter the dark compartment was recorded and used as the baseline. Twenty-four hours later, the mouse was again placed in the light compartment, and the guillotine door was opened. The time from being placed in the apparatus to the time to enter the dark compartment was recorded. The passive avoidance score (test day latency minus baseline latency) was used as an index of contextual memory formation.

Data were analyzed using an unpaired Student's *t*-test to compare individual scores.

Spatial discrimination test (T-maze). The T-maze consisted of a start box (15.3 cm × 17.6 cm) connected to a choice point area (12.2 cm × 18.0 cm) through an arm (72.5 cm long) made from a PVC pipe (5 cm diameter). This choice point in turn led to right and left goal boxes (15.3 cm × 17.6 cm) through right and left arms (72.5 cm long), respectively, also made from PVC pipes (5 cm diameter). Manually operated gates separated the choice point area from these arms. The walls of the arms and the goal boxes were decorated with visual cues, and a round cue light indicated the goal box that contained the reinforcing sucrose reward. Photobeams were positioned at five different locations within the maze (cf. Supplementary Figure S3) to record the following parameters: (a) latency to enter the first arm from the start box (photobeam located 2 cm into the arm from the start box); (b) latency to enter the choice point (photobeam located 2 cm immediately before the decision point); (c) latency to enter the goal arms; (d) latency to reach the reinforced location; and (e) latency to reach the non-reinforced location. The time between each beam break was recorded using Med Associates equipment (St Albans, Vermont) (see Supplementary Materials and Methods; Supplementary Figure S3 for further details).

Data were analyzed using a two-way repeated measures ANOVA (genotype × trial) for all dependent variables (ie, the number of correct trials completed each day and the various latency parameters listed above). *Post hoc* analysis was conducted using Fisher's LSD *post hoc* test for multiple comparisons.

Long-Term Potentiation

Preparation of hippocampal slices. Mice were anesthetized with isofluorane and decapitated. The brain was removed and placed in a cold (4°C), oxygenated (95% O_2/5% CO_2) physiological solution, ie artificial CSF (aCSF) containing (in mM) 126 NaCl, 2.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 10 glucose. The osmolarity of the aCSF was adjusted to 300 mOsm and the pH to 7.2. A block containing the hippocampus was prepared, and 300 μm coronal sections were obtained with a vibrating microtome (Leica VT 1000S, Germany). The slices were stored for 1 h in an oxygenated chamber at room temperature before they were used for the experiments.

Recording procedure. For recording, the slices were transferred to a submerged-type recording chamber and held securely in place by a nylon mesh. Oxygenated, warmed (32–34°C) aCSF was continuously superfused at a rate of 2.5 ml/min. Current- and voltage-clamp recordings were obtained with a Multiclamp 700A amplifier (Axon Instruments, Foster City, CA) under visual control using differential interference contrast and infrared video microscopy (IR-DIC; Leica DMLFSA, Germany) (see Supplementary Materials and Methods for further details).

For data analysis, the peak excitatory postsynaptic current (EPSC) amplitude was measured with respect to its baseline a few milliseconds before the stimulus artifact. Before induction, an initial baseline of current data lasting from 10 to 20 min was collected. Responses were monitored for at least 40 min after the initiation of the pairing protocol. Sample traces in the summary graphs are 5-min averages taken just before induction and between 10 and 15 min after induction. The normalized values from all mice were used to calculate ± SEM. LTP magnitude was calculated from the averaged data 10 min before induction *vs* 20 to 30 min postinduction. The same time windows were used for statistical comparisons using a two-tailed, unpaired Student's *t*-test ($p<0.01$).

RESULTS

Hippocampal KYNA and Glutamate

Compared with WT controls, KAT II KO mice showed a 71% reduction in hippocampal KYNA levels (Figure 1a) [$F(\text{genotype}) = 44.08$, $df = 31$; $p = 0.0007$]. This decrease in tissue levels was paralleled by a substantial reduction in the ambient extracellular KYNA concentration (by 66%), determined by *in vivo* microdialysis (Figure 1b) [$F(\text{genotype}) = 52.95$, $df = 1,10$; $p = 0.0001$]. Measured in the same dialysates, extracellular glutamate levels were significantly elevated in the mutant mice (by 51%) (Figure 1c) [$F(\text{genotype}) = 14.11$, $df = 1,10$; $p = 0.0037$].

Behavioral Tasks

Object exploration and recognition.

Locomotor activity component (open field): To rule out a confounding function of altered ambulatory activity in cognitive tests, we characterized locomotor behavior in an empty arena in the first session (S1) of the object exploration and recognition test. No differences in overall activity, as determined by the number of sector crossings, were found between the two genotypes (Figure 2a).

Habituation to environment (object exploration): After session S1, object exploration was assessed over three sessions (S2–S4) in which mice were allowed to freely

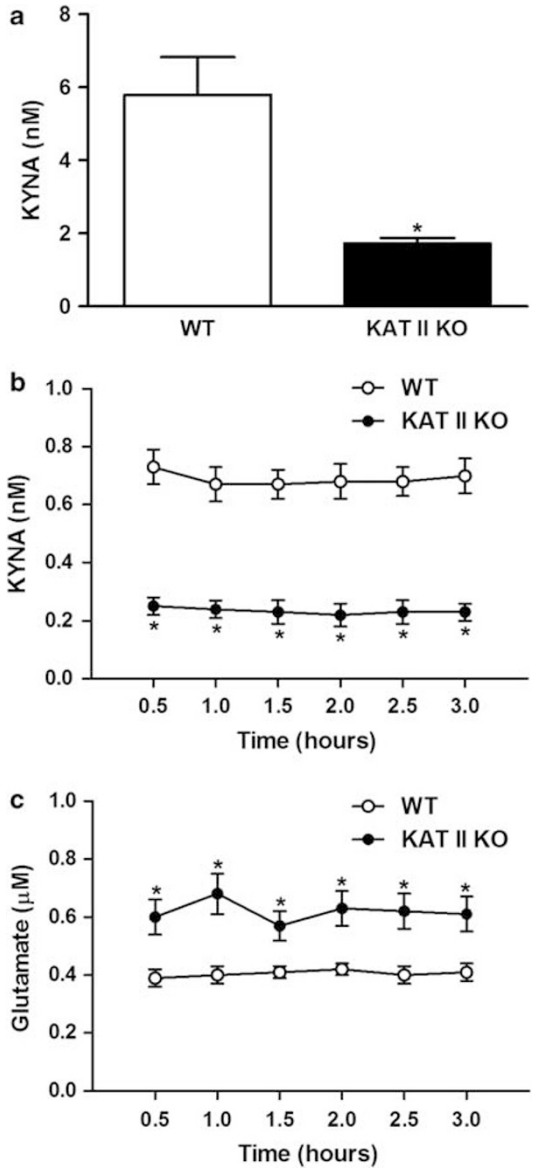

Figure 1 KYNA and glutamate in the hippocampus of KAT II knock-out (KO) mice and age-matched wild-type (WT) animals. (a) Tissue levels of KYNA in KAT II KO ($N=16$) and WT ($N=17$) mice. Data are the mean ± SEM. *$p<0.05$ *vs* WT controls; basal extracellular KYNA (b) and glutamate (c) levels, respectively, in KAT II KO mice and WT mice, measured by *in vivo* microdialysis. Data are the mean ± SEM ($N=6$ per group). *$p<0.05$ *vs* WT controls.

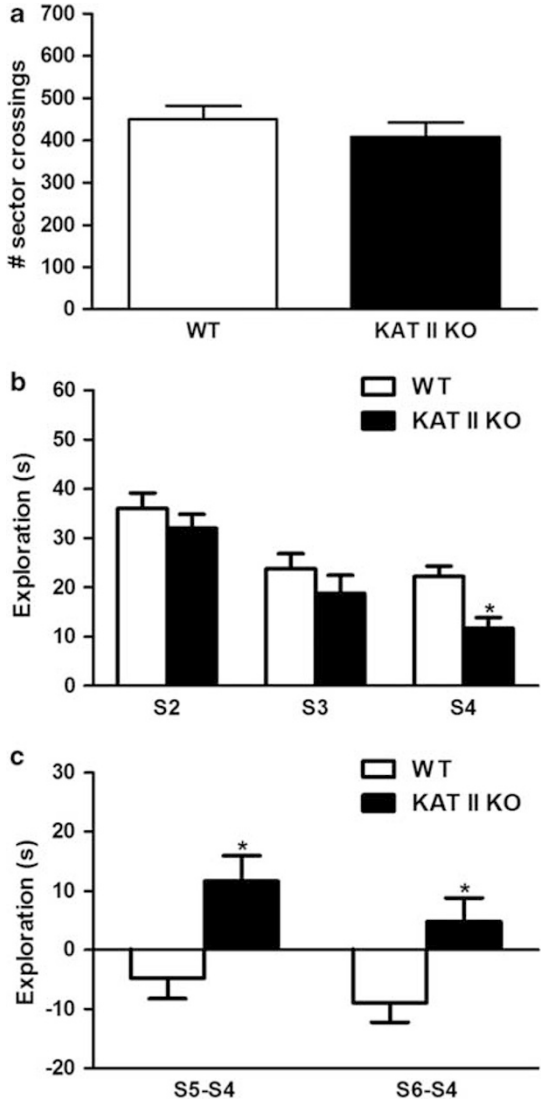

Figure 2 Improved performance of KAT II knock-out (KO) mice in an object exploration and recognition paradigm. (a) Locomotor activity (open field) and (b) object exploration (habituation to environment). Mutant mice habituated at a faster rate; (c) response to spatial change (object displacement). Compared with the baseline established in S4, KAT II KO animals spent more time than WT mice exploring the displaced object (DO) in sessions S5 and S6. Negative values reflect continued habituation in 21-day-old wild-type (WT) mice. Data are the mean ± SEM of 16 animals per group. *$p<0.05$ *vs* WT controls.

explore five different objects, which remained in the same position (Supplementary Materials and Methods; Supplementary Figure S2). The average exploration time spent at each object was recorded during these sessions. Habituation was observed in both genotypes [WT: F(session) = 1.2, df = 2,14; $p=0.0045$; KAT II KO: F(session) = 6.3, df = 2,14; $p=0.0001$]; however, mutant mice habituated at a faster rate, as evidenced by an overall main effect of genotype [F(genotype) = 4.1, df = 1,30; $p=0.0521$]. *Post hoc* analysis also revealed a significant difference between genotypes in session S4 ($p=0.0015$) (Figure 2b).

Response to spatial change (object displacement): To assess the animals' ability to recognize a change in spatial configuration, two of the five objects were moved to new locations, and the average of the exploration time spent at each of these DOs was recorded during sessions S5 and S6 (cf. Supplementary Figure S2). Exploration of the DOs was related to the respective baseline values established during session S4 (ie, S5 minus S4 and S6 minus S4). KAT II KO mice showed increased exploration of the DOs in session S5 and then habituated slightly during session S6. In contrast, WT mice did not pay disproportionate attention to the DOs and continued to decrease the amount of exploration compared with session S4 (Figure 2c). Overall, there was a significant main effect of genotype on the recognition of a change in spatial configuration [F(genotype) = 11.9, df = 1,30;

$p = 0.0017$]. *Post hoc* analysis confirmed a significant difference between genotypes on each day ($p = 0.0062$ and 0.0118, respectively).

Response to a NO (object recognition): Session S7 consisted of replacing one of the objects with a NO (cf. Supplementary Figure S2). Both genotypes explored the NO significantly more than the objects that were present during the earlier sessions (DOs and non-DOs) [WT: F(object) = 7.4, df = 1,15; $p = 0.0157$; KAT II KO: F(object) = 4.9, df = 1,15; $p = 0.0426$]. The ratio of time spent exploring the NO *vs* the average time spent exploring the non-DOs was greater in the KAT II KO mice (WT: 1.6 ± 0.6; KAT II KO: 2.4 ± 0.6); however, this difference was not statistically significant. These results suggest that the NO, in contrast to the DOs in session S5 and session S6, constituted a strong, salient stimulus, which prevented detection of differences between the two genotypes.

Contextual memory (passive avoidance). Contextual memory was assessed in a two-chamber (light/dark) passive avoidance paradigm. Mice were tested for their ability to remember, over a 24-h period, the context in which an aversive stimulus (shock) was delivered. In this test, the animals are required to inhibit their natural tendency to leave a brightly lit chamber and move into a dark chamber. KAT II KO mice performed better than WT controls, as evidenced by a significantly longer latency to enter the dark side of the chamber that had earlier resulted in shock delivery [F(genotype) = 1.224, df = 31; $p = 0.0009$] (Figure 3a). We observed no genotype difference in the physical reaction to the shock.

Spatial discrimination test (T-maze). Mice were trained in a spatial discrimination task to locate the goal box containing a reinforcing sucrose reward in a T-maze (cf. Supplementary Figure S3). WT and KAT II KO mice began the training process at equal performance levels. Overall, KAT II KO mice then made significantly more correct choices than WT mice [F(genotype) = 14.93, df = 1,32; $p = 0.0005$]. *Post hoc* analysis revealed genotypic differences beginning on day 4 until performance in WT and KAT II KO mice became statistically indistinguishable on day 10 (Fisher's LSD *post hoc* test for multiple comparisons) (Figure 3b). Moreover, mutant mice took significantly less time to select a maze arm once they reached the choice point of the apparatus [F(genotype) = 10.46, df = 1,32; $p = 0.003$] (Figure 3c). The genotype difference was not due to motivational deficits, as evidenced by comparable latencies to enter the start arm, or to motor deficits, as the two groups showed similar latencies to reach the choice point from the start box, the reinforced location, and the non-reinforced location (Supplementary Figure S4).

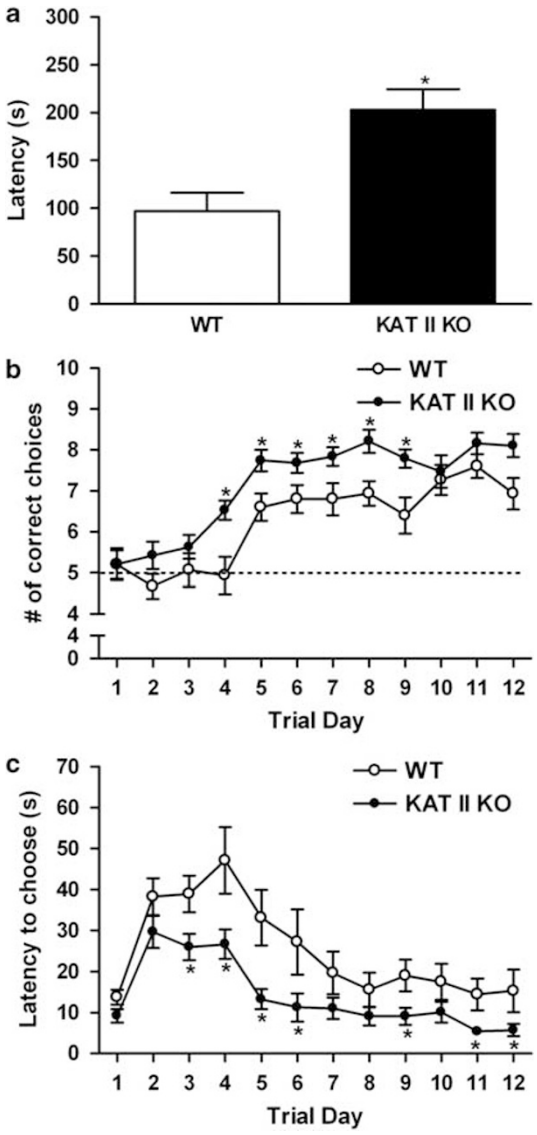

Figure 3 Improved performance of KAT II knock-out (KO) mice in passive avoidance (contextual memory) and spatial discrimination (T-maze) tests. (a) Performance of KAT II KO and wild-type (WT) mice in a passive avoidance paradigm. Latency is defined as the difference between the times taken to enter the dark compartment on the two test days. Data are the mean ± SEM of 16 KAT II KO and 17 WT mice. $^*p < 0.05$ *vs* WT controls. (b) Number of correct choices (to locate a sucrose reward from a goal box) as a function of training days in a T-maze. Mice were 21 days old on the first day of the trial. Five correct choices represent chance (dashed line). (c) Latency to choose either the reinforced or the non-reinforced arm. Data in (b, c) are the mean ± SEM of 19 KAT II KO and 15 WT mice. $^*p < 0.05$ *vs* WT controls.

Long-Term Potentiation

In the Schaffer collateral/commissural synapse onto CA1 pyramidal cells, LTP is Hebbian, requiring NMDAR activation and elevation of postsynaptic calcium (Bliss and Lømo, 1973; Cummings *et al*, 1996). LTP was induced in CA1 pyramidal cells using a pairing protocol consisting of three brief high-frequency tetani (50 pulses at 100 Hz, 4 s intervals), applied at the end of a 3 min depolarization at 0 mV. Slices obtained from WT mice showed an increase of $126 \pm 12\%$ ($N = 12$; $p < 0.05$) above the baseline of synaptic responses, lasting for more than 40 min (Figure 4a). This LTP was NMDAR-dependent as it was prevented by DL-2-amino-5-phosphonovaleric acid (AP-5, 50 μM; $-10 \pm 9\%$ of baseline; $N = 5$; Figure 4a). Using the same protocol, we observed a substantial increase in the amplitude of LTP in

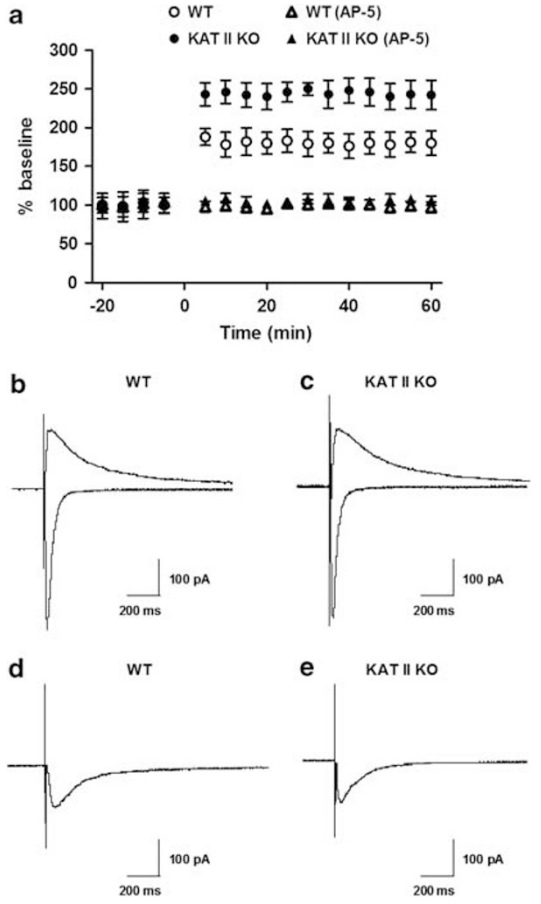

Figure 4 Enhanced LTP amplitude in hippocampal slices obtained from 21–28-day-old KAT II knock-out (KO) and age-matched wild-type (WT) mice. (a) All values are expressed relative to the baseline, ie the average of the responses during a 10 min period before LTP induction. Each point represents the average of the responses recorded in 60 s. Application of the NMDAR antagonist AP-5 (50 μM) blocked LTP in slices from either genotype. Data are the mean ± SEM (12 slices per group). *$p<0.05$ *vs* WT controls. No significant genotypic differences were observed in NMDAR properties (resting membrane potential, firing pattern, and action potential amplitude) (b, c) or the AMPAR/NMDAR ratio (using average peak EPSCs at +40 mV) (d, e).

slices from mutant mice (248 ± 21% above baseline; $N=12$; $p<0.05$). The enhanced LTP in mutant mice was significantly greater than in WT controls ($p<0.005$) and was also prevented by 50 μM AP-5 (Figure 4a).

Next, we compared additional electrophysiological characteristics of CA1 pyramidal cells in WT and KAT II KO animals. Current-clamp experiments, performed using aCSF containing low Mg^{2+} (0.1 mM), bicuculline (20 μM), picrotoxin (50 μM), CGP-52532 (10 μM), strychnine (2 μM), and NBQX (10 μM) (Bergeron *et al*, 2007), did not reveal significant genotypic differences in terms of resting membrane potential, firing pattern, or action potential amplitude (Figure 4b and c). We then measured the decay kinetics of NMDARs using the average of 25 traces and the double exponential function $y=A_f e^{-t/\tau}+A_s e^{-t/\tau}$, where A is the amplitude, τ is the decay time constant, and the subscripts f and s denote fast and slow components, respectively. These studies failed to show a significant genotypic difference in the 10–90% rise time of NMDAR EPSCs (WT: 23.7 ± 3.1 ms, KAT II KO: 24.5 ± 3.9 ms; $N=6$ each). Moreover, no significant differences were observed in the averaged τ_s and τ_f values of mutant and WT mice.

In a third study, we determined the AMPAR/NMDAR ratios, using the average peak EPSCs at +40 mV in aCSF containing picrotoxin, CGP 52432, and strychnine. The AMPAR-mediated current was defined as the difference between the responses seen in the absence and presence of NBQX. These experiments revealed no significant genotypic difference (Figure 4d and e).

DISCUSSION

We used mice with a genomic deletion of KAT II, a major enzyme of KYNA biosynthesis in the mammalian brain (Guidetti *et al*, 2007a), to test the hypothesis that a reduction in brain KYNA levels enhances cognitive function. Focusing on the hippocampus because of its well-recognized role in cognitive processes (Morris, 2006), we used several *in vivo* approaches, including the measurement of extracellular glutamate levels and performance in three behavioral tasks, and also assessed LTP in tissue slices *ex vivo*. Both individually and jointly, our data indicated a close association between a reduction in KYNA formation and cognitive enhancement. More generally, and similar to the conceptually related proposition of Pittaluga *et al* (1997), we, therefore, suggest that a decrease in cerebral KYNA synthesis may constitute a biological mechanism that results in improved cognitive performance.

The behavioral paradigms (object exploration and recognition, passive avoidance, and spatial discrimination) were chosen for their ability to probe cognitive function that relies, to a considerable extent, on hippocampus-dependent circuitry. In particular, the object exploration and recognition test is an innately driven multidimensional task that requires the subject to sequentially build a spatial representation of the environment and use it to recognize change; the contextual memory (passive avoidance) task necessitates the initial consolidation and subsequent retrieval of an aversive-event memory over a 24-h period; and the spatial discrimination task involves discriminative spatial choice and reference memory to approach and obtain a reward (Save *et al*, 1992; Schimanski and Nguyen, 2004; Robinson *et al*, 2005).

Experiments in cultured cells and tissue slices, as well as *in vivo* studies in animals and human beings, have documented the central function of glutamate in learning and memory. Although the identity and precise functions of the ionotropic and metabotropic glutamate receptors involved, as well as the complex inter- and intracellular processes triggered by their activation, are still being elaborated, there is general consensus that increased glutamatergic activity promotes the establishment of the permanent synaptic changes that are necessary for the acquisition or recall of information (Bannerman *et al*, 2006; Robbins and Murphy, 2006). This conclusion is based mainly on pharmacological studies in a large number of test systems, including the experimental endpoints studied here, showing cognitive impairments after animals are treated

with glutamate receptor *antagonists* (Hauber and Schmidt, 1989; Venable and Kelly, 1990; Karasawa *et al*, 2008).

Pro-cognitive effects of glutamate receptor activation are more difficult to observe, though improved performance has been described under normal physiological conditions when glutamatergic activity is stimulated by endogenous or exogenous receptor agonists (Lynch and Gall, 2006; Clem *et al*, 2008; Singer *et al*, 2009). Of relevance here, increased glutamatergic tone enhances memory function in relevant model systems (Richter-Levin *et al*, 1995; Uslaner *et al*, 2009). The present study in KAT II KO mice, which shows that a decrease in KYNA levels has the same functional consequences as direct glutamate receptor stimulation, is not only consistent with this concept, but introduces reduced KYNA formation as a novel endogenous control mechanism of cognitive behavior.

The higher ambient levels of extracellular glutamate in KAT II KO mice appear to be a direct consequence of reduced KYNA synthesis. On the basis of anatomical and pharmacological studies, it is likely that this link between KYNA and glutamate involves α7nAChRs. Thus, in the hippocampus as in other brain regions, glutamatergic nerve terminals are richly endowed with α7nAChRs, which control neuronal glutamate release (Gray *et al*, 1996; Girod *et al*, 2000; Grilli *et al*, 2006); and α7nAChR agonists enhance, whereas α7nAChR antagonists reduce, the liberation of glutamate into the extracellular milieu (Carpenedo *et al*, 2001; Rassoulpour *et al*, 2005; Wang *et al*, 2006; Lagostena *et al*, 2008). Of importance in the context of this study, the *in vivo* effects of classic α7nAChR antagonists are duplicated by KYNA (Carpenedo *et al*, 2001; Rassoulpour *et al*, 2005; Lopes *et al*, 2007), and the KYNA-induced reduction in glutamate release is neutralized by the co-administration of an α7nAChR agonist (Rassoulpour *et al*, 2005). Moreover, the KYNA concentrations applied in these and similar *in vivo* studies—using KYNA-induced reductions in dopamine and acetylcholine as experimental end points (Rassoulpour *et al*, 2005; Zmarowski *et al*, 2009)—are in the endogenous, ie low- to mid-nanomolar range (cf. also Grilli *et al*, 2006). These concentrations are far below those required to inhibit glutamate receptors directly (Perkins and Stone, 1982; Kessler *et al*, 1989), especially in the presence of endogenous glycine (Hilmas *et al*, 2001). Thus, we assume that fluctuations in endogenous KYNA levels tonically modulate α7nAChR function and glutamate release *in vivo*. The fact that selective α7nAChR activation enhances LTP in the CA1 region (Lagostena *et al*, 2008) and that several α7nAChR agonists reliably show pro-cognitive effects in established behavioral models including those used in this study, indirectly underscores the functional importance of the link between nicotinic stimulation and glutamatergic acitivity (Levin *et al*, 2006; Albuquerque *et al*, 2009; Roncarati *et al*, 2009).

Our results do not rule out additional glutamatergic adaptations in KAT II KO mice. Thus, the significantly higher amplitude response in mutant mice compared with WT controls, which was observed in our study of NMDAR-dependent LTP, might have been caused by genotypic differences in the probability of glutamate release, the efficiency of neuronal glutamate transporters, and/or the function of proximal astrocytes (Filosa *et al*, 2009). Notably, however, we ruled out the most parsimonious mechanism, namely changes in the number or ratio of postsynaptic AMPARs and NMDARs in KAT II KO mice.

In addition to its emerging function in brain physiology, abnormal KYNA levels and function have been speculatively linked to several human brain disorders with cognitive symptomatology (Schwarcz and Pellicciari, 2002; Sapko *et al*, 2006; Raison *et al*, 2009; Vámos *et al*, 2009). In light of the present results, KYNA might be of special relevance to the pathophysiology of schizophrenia. As mentioned earlier (cf. Introduction), brain and CSF KYNA levels are increased in the disease, and elevated brain KYNA levels in animals cause impairments that are reminiscent of the cognitive deficits seen in patients (Shepard *et al*, 2003; Erhardt *et al*, 2004; Chess and Bucci, 2006; Chess *et al*, 2007). By inhibiting α7nAChRs and glutamate release, enhanced KYNA may, therefore, contribute to the hyponicotinergic and hypoglutamatergic tone that is believed to underlie the cognitive deficits seen in patients (Buchanan *et al*, 2007). It follows that interventions targeting kynurenine pathway metabolism to decrease KYNA synthesis in the brain may not only be effective under normal physiological conditions, but may be especially valuable for improving cognition in individuals with schizophrenia (Schwarcz and Pellicciari, 2002).

The simplest approach to lower cerebral KYNA levels in health or disease is to target the enzymatic synthesis of KYNA. Although the mammalian brain contains several aminotransferases capable of producing KYNA from its immediate bioprecursor kynurenine (Guidetti *et al*, 2007a; Han *et al*, 2009), KAT II is the most substrate specific—and, therefore, 'druggable'—of these enzymes. Moreover, only KAT II has so far been conclusively linked to brain physiology (Amori *et al*, 2009; Zmarowski *et al*, 2009; Wu *et al*, 2010), increased vulnerability to massive excitotoxic insults (Sapko *et al*, 2006), and, as shown in this study, cognitive function. Selective inhibition of KAT II, therefore, appears to constitute the best means to attenuate KYNA formation and at the same time enhance cognition under either physiological or pathological conditions. In light of this study, novel KAT II inhibitors with good blood–brain barrier penetration will hopefully soon take the place of first-generation compounds, which do not enter the brain after peripheral administration (Pellicciari *et al*, 2006).

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DISCLOSURE

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