A runner's high depends on cannabinoid receptors in mice

Johannes Fuss[a,b,1,2], Jörg Steinle[a,1], Laura Bindila[c], Matthias K. Auer[d], Hartmut Kirchherr[e], Beat Lutz[c], and Peter Gass[a]

[a]Department of Psychiatry and Psychotherapy, Central Institute of Mental Health, University Medicine Mannheim, University of Heidelberg, 68159 Mannheim, Germany; [b]Institute for Sex Research and Forensic Psychiatry, Center of Psychosocial Medicine, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany; [c]Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University Mainz, 55099 Mainz, Germany; [d]Department of Clinical Neuroendocrinology, Max Planck Institute of Psychiatry, 80804 Munich, Germany; and [e]Medizinisches Labor Bremen, 28357 Bremen, Germany

Edited by Leslie Lars Iversen, University of Oxford, Oxford, United Kingdom, and approved September 4, 2015 (received for review July 29, 2015)

Exercise is rewarding, and long-distance runners have described a runner's high as a sudden pleasant feeling of euphoria, anxiolysis, sedation, and analgesia. A popular belief has been that endogenous endorphins mediate these beneficial effects. However, running exercise increases blood levels of both β-endorphin (an opioid) and anandamide (an endocannabinoid). Using a combination of pharmacologic, molecular genetic, and behavioral studies in mice, we demonstrate that cannabinoid receptors mediate acute anxiolysis and analgesia after running. We show that anxiolysis depends on intact cannabinoid receptor 1 (CB1) receptors on forebrain GABAergic neurons and pain reduction on activation of peripheral CB1 and CB2 receptors. We thus demonstrate that the endocannabinoid system is crucial for two main aspects of a runner's high. Sedation, in contrast, was not influenced by cannabinoid or opioid receptor blockage, and euphoria cannot be studied in mouse models.

endocannabinoid | running | exercise | anxiety | anandamide

A runner's high is described as an ephemeral pleasant phenomenon that may be experienced during long-term running. A popular belief has been that endorphins mediate a runner's high, although neurobiological mechanisms were unclear. In earlier experiments, two prominent systems (the opioid and endocannabinoid systems) were suggested to be involved in a runner's high (1–3). Running increases plasma levels of β-endorphin (an opioid) and anandamide (an endocannabinoid) in mice and men (4, 5). However, unlike the lipophilic anandamide, β-endorphin cannot cross the blood–brain barrier, rendering central effects of peripheral opioids unlikely. In an attempt to disentangle the biomechanism of a runner's high, we were using a combination of pharmacologic, molecular genetic, and behavioral studies in mice, and demonstrated for the first time to our knowledge that a runner's high depends on cannabinoid receptors in mice.

Results and Discussion

In a first step, mice ($n = 32$) were provided with running wheels for 3 d to start with and to habituate them to wheel running. Mice ran, on average, 5.4 km per day (Fig. 1*A*). After 2 d with blocked running wheels, half of the mice were assigned into a running (RUN) and the other half to a nonrunning (CON) group, considering matched running distances. Runners ($n = 16$) were again subjected to a brief period of wheel running (5 h) directly before behavioral testing (day 6) and ran, on average, 6.5 ± 0.7 km (Fig. 1*A*).

When subsequently tested for anxiety-like behavior in the dark–light box test, runners exhibited significantly less anxiety by spending an increased time in the aversive bright area than controls ($P = 0.002$; Fig. 1*B*). Runners were also less active and displayed fewer exits from the dark compartment into the lit compartment (RUN, 10.3 ± 0.8 exits; CON, 12.6 ± 0.7 exits; $P = 0.040$). Next, mice were removed from the dark–light arena and subjected to the hot plate test to study pain sensitivity. Here, runners displayed an increased latency to lick hind paws or jump (first action), suggesting reduced thermal pain sensitivity ($P = 0.024$; Fig. 1*C*). After the behavioral testing, mice were returned to their home cage, and all mice received free wheel access for 1 h. Controls now ran significantly more than mice of the running group (RUN, 0.28 ± 0.06 km; CON, 0.55 ± 0.08 km; $P = 0.008$). We were thus able to demonstrate that acute long-distance running reduces anxiety and pain. Runners were also less active when exposed to running wheels after behavioral testing, which indicates postexercise sedation. Thus, three of the four features (anxiolysis, analgesia, and sedation) of a runner's high were observable in mice. However, the fourth feature, euphoria, is a highly subjective feeling that may be difficult to model in mice. Two days after behavioral testing, half of the mice were again subjected to 5 h of wheel running, and endocannabinoids (eCBs) in plasma, cerebrospinal fluid (CSF), and various body tissues were measured. Running significantly elevated eCBs in plasma [anandamide (AEA), $P = 0.03$; 2-arachidonoglycerol (2-AG), $P = 0.12$; palmitoylethanolamide (PEA), $P = 0.09$; oleoylethanolamide (OEA), $P = 0.001$; and arachidonic acid (AA), $P < 0.001$; Fig. 1*D*] without affecting eCBs in CSF or various other tissues (Table S1). Of note, eCB levels in various brain regions were also not affected by acute long-distance running. In contrast, a recent study found that chronic wheel running seems to increase anandamide levels in the hippocampus of rodents (6). In general, the lipophilic structure of eCBs may affect the accurate detection of subtle changes in eCB levels in brain regions as a result of their fast elimination and distribution.

We next aimed to pharmacologically dissect whether eCBs or endorphins mediate acute running-induced anxiolysis and analgesia.

Significance

A runner's high is a subjective sense of well-being some humans experience after prolonged exercise. For decades, it was hypothesized that exercise-induced endorphin release is solely responsible for a runner's high, but recent evidence has suggested that endocannabinoids also may play a role. Here, we demonstrate that wheel running increases endocannabinoids and reduces both anxiety and sensation of pain in mice. Ablation of cannabinoid receptor 1 receptors on GABAergic neurons inhibits running-induced anxiolysis, and pharmacological blockage of central and peripheral cannabinoid receptors inhibits analgesia. We thus show for the first time to our knowledge that cannabinoid receptors are crucial for main aspects of a runner's high.

Author contributions: J.F. and P.G. designed research; J.F., J.S., and L.B. performed research; L.B., M.K.A., H.K., and B.L. contributed new reagents/analytic tools; J.F. and J.S. analyzed data; and J.F., B.L., and P.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]J.F. and J.S. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: jo.fuss@uke.de.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1514996112/-/DCSupplemental.

NEUROSCIENCE

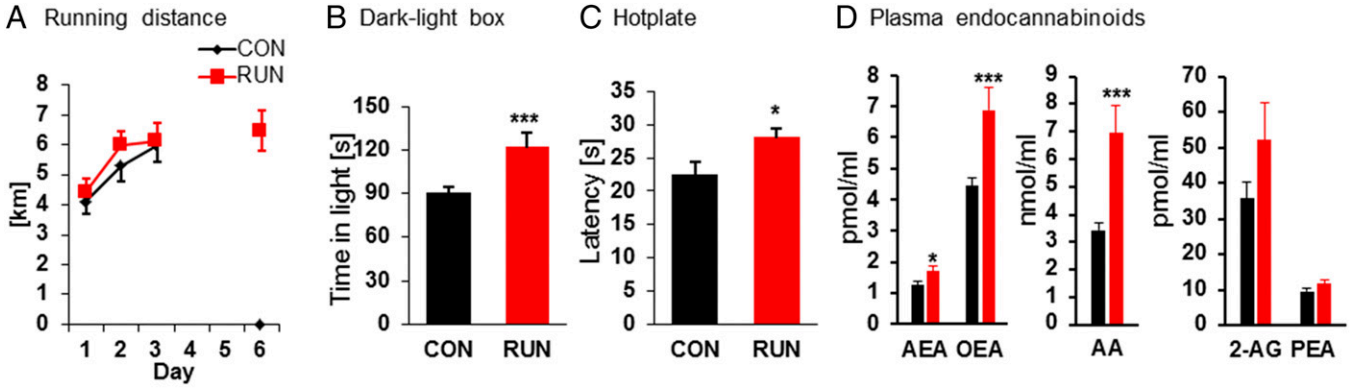

Fig. 1. The effects of acute long-distance running in mice. All mice had access to running wheels for 3 d (*A*). After 2 d without wheels, half of the mice (RUN) were given again free access for 6 h, whereas controls (CON) remained sedentary. Mice were behaviorally tested subsequently, and RUN exhibited reduced anxiety-like behavior (*B*) and reduced thermal pain sensitivity (*C*). eCBs were increased after running (*D*). $N = 16$ controls and $n = 16$ runners for *A*–*C*, and $n = 13$ controls and $n = 8$ runners for *D*. *Significant difference, with $P < 0.05$. ***$P < 0.001$. Columns represent means + SE.

We therefore repeated the first experiment; however, mice were injected with eCB- or endorphin-antagonists (or vehicle) before running (i.e., 5 h before behavioral testing) on day 6. The subsequent running performance was not affected by treatment ($F_{5,71} = 1.072$; $P = 0.38$). In contrast, a two-factorial ANOVA again demonstrated a significant effect of running on anxiety-like behavior in sham-treated mice ($F_{1,134} = 17.70$; $P < 0.001$; Fig. 2*A*). Thus, despite the i.p. injection, we once again observed anxiolysis, as in the first experiment. Post hoc comparisons, however, revealed that central cannabinoid receptor 1 (CB1) blockage via the inverse agonist AM251 [3 mg/kg body weight (kgBW) ($n = 26$; $P = 0.96$); 1 mg/kgBW ($n = 28$, $P = 0.20$)] was sufficient to inhibit the running-induced anxiolytic phenotype. In contrast, in all other treatment groups, the anxiolytic phenotype persisted, despite inhibition of endorphin signaling via naloxone (2 mg/kgBW; $n = 20$; $P = 0.005$) or blockage of CB2 receptors via AM630 (3 mg/kgBW; $n = 26$; $P = 0.03$). A trend was observed after blockage of peripheral CB1 receptors via AM6545 (3 mg/kgBW; $n = 26$; $P = 0.1$).

In the hot plate test, the running-induced reduction of thermal pain sensitivity was absent after both peripheral and central antagonism of CB1 and CB2 receptors, respectively. We found a significant effect of treatment ($F_{5,134} = 16.69$; $P < 0.001$) and running ($F_{1,134} = 8.34$; $P = 0.005$), and an interaction of running and treatment ($F_{5,134} = 3.37$; $P = 0.007$). Although running mice that received vehicle ($P < 0.001$) or naloxone ($P = 0.004$) exhibited increased latencies to react to the hot plate compared with non-running controls, AM6545 ($P = 0.79$), AM251 (1 mg/kgBW, $P = 0.47$; 3 mg/kgBW, $P = 0.91$), and AM630 ($P = 0.52$) inhibited the effect of running on thermal pain sensitivity. Thus, analgesia seems to be also mediated by peripheral CB1 and CB2 receptors.

In line with our findings, a recent study found that the eCB system mediates exercise-induced antinociception at the peripheral and central levels (7). After behavioral testing, all mice were again subjected to free wheel access, and similar to in experiment 1, controls ran significantly longer distances ($F_{1,133} = 18.17$; $P < 0.001$). Thus, pharmacological treatments with cannabinoid receptor antagonists did not affect running performance after behavioral testing ($F_{5,133} = 1.79$; $P = 0.12$). This finding speaks against a mechanistic role of eCBs in the runner's high sedation. Possibly, the sedation observed after 5 h of running could also result from running-induced exhaustion.

Because our pharmacological experiments indicated that selective blockade of central CB1 receptors is sufficient to inhibit the acute anxiolytic effect of running, we were aiming to confirm this hypothesis using a targeted mutagenesis approach. Earlier, it had been demonstrated that mice with a deletion of CB1 receptors on GABAergic neurons exhibit less wheel running activity when wheel running is restricted to 3 h per day (8). A possible reason is that these mice receive less emotional benefit from wheel running, and thus lose interest. We therefore chose this mouse model to investigate the influence of acute running on anxiety-like behavior. Because our pharmacological experiment had revealed a role for CB1 and CB2 receptors in the periphery for the reduction of pain sensitivity in the hot plate test, we omitted hot plate experiments in the present experiment. Running and anxiety testing were performed as before. First, under unrestricted conditions, GABA-$CB_1^{+/+}$ mice ($n = 28$) ran 10% more than

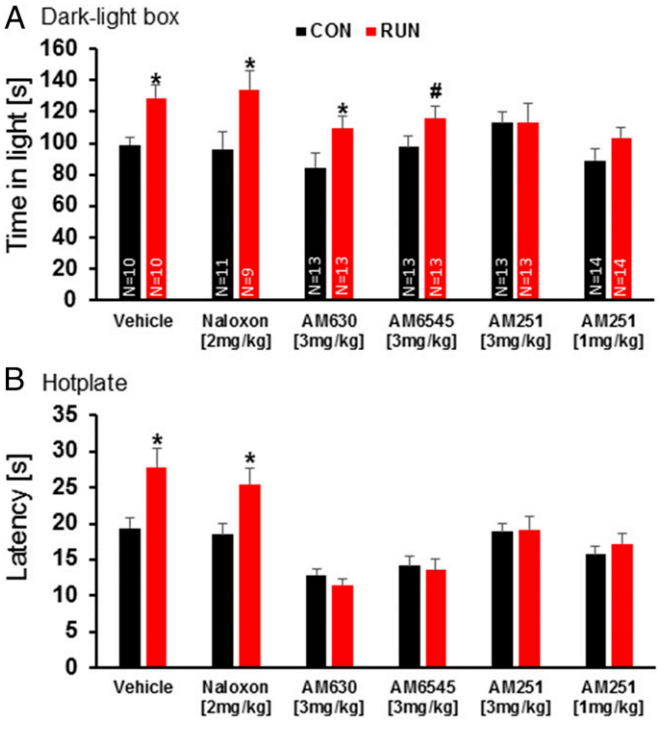

Fig. 2. Pharmacological blockage of central CB1 receptors with AM251 prevents the reduction of anxiety in runners (RUN) (*A*). The running-induced reduction of thermal pain sensitivity is absent after administration of peripheral CB1 or CB2 antagonists (*B*). *Significant difference in post hoc test. #Only a difference on trend level $P = 0.1$. The group size for both tests is indicated with N on *A*. Columns represent means + SE.

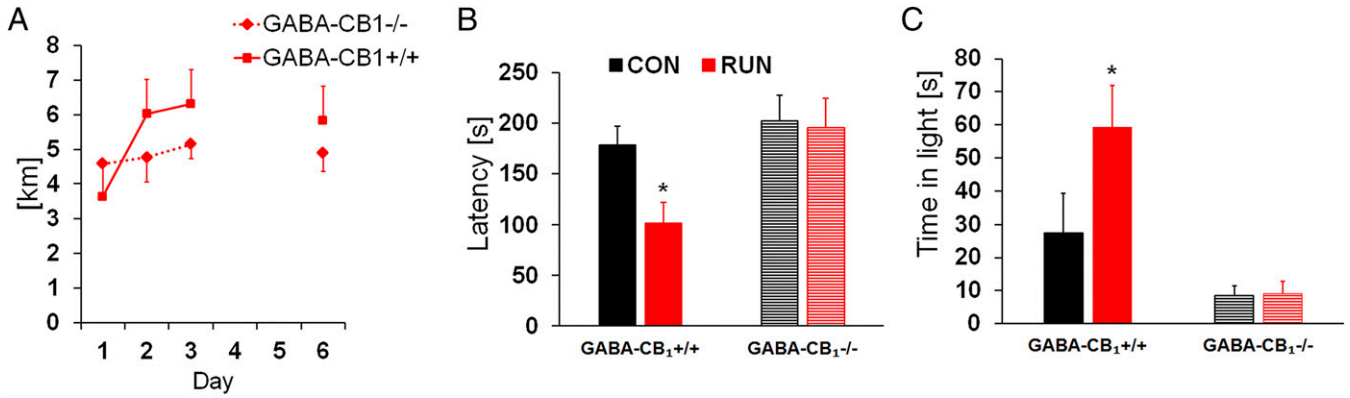

Fig. 3. Acute exercise reduces anxiety in GABA-$CB_1^{+/+}$ mice, but not in GABA- $CB_1^{-/-}$. GABA-$CB_1^{+/+}$ mice perform about 10% less wheel running before behavioral testing (*A*). In the dark–light test, running has no anxiolytic effect in mice with a conditional deletion of CB1 receptors on GABAergic neurons (*B* and *C*). $N = 14$ per group. Columns represent means + SE.

GABA-$CB_1^{-/-}$ ($n = 28$) mice during days 1–3 (GABA-$CB_1^{+/+}$, 5.3 km/d on average; GABA-$CB_1^{-/-}$, 4.8 km/d on average; $P = 0.48$) and on day 6 (GABA-$CB_1^{+/+}$, 5.8 km; GABA-$CB_1^{-/-}$, 4.9 km; $P = 0.27$; Fig. 3*A*); however, the difference was not statistically significant, in contrast to the study by Dubreucq and colleagues (8).

In the dark–light box, two-way ANOVA revealed a significant effect of genotype [latency, $F_{1,52} = 6.21$ ($P = 0.02$); time in lit compartment, $F_{1,52} = 14.63$ ($P < 0.001$)] and a trend for running [latency, $F_{1,52} = 3.11$ ($P = 0.08$); time in lit compartment, $F_{1,52} = 3.23$ ($P = 0.07$)]. As could be expected from our earlier experiments, post hoc comparisons revealed that anxiety-like behavior was reduced in GABA-$CB_1^{+/+}$ runners (wild-types). Thus, the latency to enter the aversive lit compartment ($P = 0.03$; Fig. 3*B*) was shortened in runners. Running GABA-$CB_1^{+/+}$ mice furthermore spent significantly more time in the aversive lit compartment ($P = 0.02$; Fig. 3*C*). In contrast, in GABA-$CB_1^{-/-}$ mice, we observed no effect of acute running on anxiety-like behavior, demonstrating that deletion of CB1 receptors on GABAergic neurons is sufficient to block the running-induced anxiolytic phenotype. Similar to our pharmacological experiments with CB1 receptor antagonists, running performance after the test was again affected by pretest running ($F_{1,51} = 4.35$; $P = 0.04$), but not by genotype ($F_{1,51} = 0.07$; $P = 0.79$).

In conclusion, although a biomechanism for the beneficial effects of chronic long-term running was reported more than 2 decades ago (9–11), we report here how acute long-distance running reduces anxiety-like behavior and induces analgesia and sedation in mice. These acute effects of running, together with a feeling of euphoria, were earlier termed a runner's high in humans. In a series of experiments, we were able to show that the reduction in anxiety-like behavior after acute long-distance running depends on CB1 receptors on forebrain GABAergic neurons. Pain reduction, in contrast, depends on peripheral CB1 and CB2 receptors. Our data demonstrate that an intact eCB system is crucial for a runner's high in mice.

Materials and Methods

Animals. We performed three experiments with 234 male mice altogether. For experiments 1 and 2, male C57BL/6J mice ($n = 178$) were obtained from Charles River at 8 wk of age. In the first experiment ($n = 32$), we studied how acute running affects anxiety-like behavior and thermal pain sensitivity. In the second experiment ($n = 146$), we were aiming to block the observed running-induced phenotype of experiment 1 by pharmacological antagonism of endorphin and eCB signaling. For experiment 3, male C57BL/6N wild-type ($n = 28$; GABA-$CB_1^{+/+}$) and mutant mice lacking CB1 receptor specifically on forebrain GABAergic neurons ($n = 28$; GABA-$CB_1^{-/-}$) were received from University Mainz. GABA-$CB_1^{-/-}$ mice were generated, bred, and genotyped as previously described (12, 13) by using a transgenic *Dlx5/6-Cre* recombinase mouse line crossed with a CB1 receptor floxed mouse line. After their arrival, all animals were single housed in type III macrolon cages in a temperature- and humidity-controlled room on a 12-h dark–light cycle (light, 7:00 AM–7:00 PM) for at least 2 wk before starting experiments. Animals received water and food ad libitum. All experimental procedures were approved by the German animal welfare authorities.

Experimental Protocol. Two weeks after their arrival, all animals received free access to a running wheel for 3 d, as described earlier (14). The next 2 d (days 4 + 5), all wheels were blocked. Running distances during the adaption phase on days 1–3 were measured daily, and mice were subsequently assigned into a running (RUN) and a nonrunning (CON) group considering matched running distances. On day 6, all mice were conveyed to the experimental room before the beginning of the dark cycle. The running wheels of the running group were unblocked for 5 h, whereas the control group remained with blocked wheels for 5 h. Mice were than tested in the dark–light box for 5 min. The dark–light box is a behavioral test to assess anxiety-like behavior in mice. It consists of two plastic chambers connected by a small tunnel. The dark chamber measures 15×20 cm^2 and is covered by a lid. The adjacent chamber, measuring 15×30 cm^2, is white and illuminated from above with 600 Lux. Mice were placed solitarily into the dark compartment. Latency to first exit and end exploration time (i.e., the latency until the mice reached the wall at the end of the bright compartment), as well as number of exits and total time spent in light, were recorded for 5 min (15). After 5 min in the dark–light box, mice (experiment 1 + 2) were directly transferred onto the hot plate test to determine pain sensitivity (ATLab). The plate temperature was set at 53 °C (± 0.3 °C). Time of first licking hind paws or jumping was assessed with a cutoff at 45 s to prevent injury. After behavioral testing (dark–light box and hot plate for experiment 1 + 2, dark–light box for experiment 3) all mice (RUN and CON) were subjected to free access to running wheels for 1 h. Ten to 12 mice were tested per day, so we had to run several replicates.

Drug Treatment. Drug treatment groups received either vehicle or verum i.p. before starting to run on day 6 (i.e., 5 h before behavioral testing). The following drugs and concentrations were used: CB1 antagonist AM251 (3 and 1 mg/kg), opioid antagonist naloxone (2 mg/kg), CB2 antagonist AM630 (3 mg/kg), and the peripherally selective CB1 receptor antagonist AM6545 (3 mg/kg). All substances were dissolved in 5% (vol/vol) DMSO, 5% (vol/vol) Tween20, and 90% (vol/vol) saline and diluted to a volume of 10 mL/kg body weight. Controls received the same volume of vehicle. Substances were purchased from Sigma-Aldrich. Because the half-life of naloxone is much shorter than the half-life of all other substances (16–18), we performed a pharmacokinetic experiment in 27 mice to study plasma levels 3 and 6 h after injection to determine dosage of naloxone (details for this experiment can be found in Fig. S1).

Sample Analysis. In experiment 1, 26 mice were returned to home cages with blocked wheels and rested for 2 d after behavioral testing. For sample analysis, half of the running wheels were unblocked, and mice were able to perform wheel running. In the runners group, five mice performed no wheel running and were therefore omitted. After 5 h of running, mice were anesthetized by i.p. injection of ketamine and xylazine, and an occipital

lumbar puncture was performed in a stereotaxic frame with a G25 butterfly needle to receive cerebrospinal fluid. Plasma was sampled from the right heart ventricle, and the following tissues were collected and frozen on dry ice: heart, lung, skeletal muscle, liver, white fat tissue, brown fat tissue, duodenum, ileum, kidney, prefrontal cortex, hypothalamus, hippocampus, and cerebellum.

Endocannabinoid Extraction and Quantification. For eCBs extraction, tissues were first weighted in the cold room and transferred to precooled Precelly tubes or Qiagen strips containing cold ceramic or steel ball beads, respectively. Spiking solution of deuterated eCBs in acetonitrile was mixed with ethyl acetate/hexane (9:1, vol/vol) and added to the tissue samples, followed by 0.1 M formic acid. Typical parameters for homogenization were 15 s at 5,000 rpm with a Precellys 24 (Bertin Technologies) or 30 s at 30 Hz for tissue lyser (Qiagen) and then centrifuged at 10,000 × *g* and 4 °C for 10 min. The number of cycles for homogenization, as well as the volumes for extraction solvent and homogenization buffer, were tailored to the tissue type. The samples were then kept for 10 min at −20 °C to freeze the aqueous phase. The upper organic phase was recovered and evaporated to dryness, and the extracts were reconstituted in 50 μL water:acetonitrile (1:1, vol/vol) for further liquid chromatography/multiple reaction monitoring (LC/MRM) analysis. The extraction of eCBs from plasma and CSF followed essentially the same procedure, except the homogenization was replaced by vortexing.

Throughout the extraction procedure, the tubes, plates, beads, and so on were invariably precooled and kept at 4 °C. The samples were, as well, invariably kept on ice throughout the entire extraction procedure to prevent artificial alterations of endogenous eCB levels originating from enzymatic or chemical degradation and/or ex vivo synthesis of eCBs. The amounts of internal standards and concentration range of calibration curves were selected using test tissue plasma and CSF samples.

Quantification of eCBs in tissues, plasma, and CSF was carried out by LC/MRM, using the LC and MRM conditions as previously described (19). eCB concentrations were normalized to the tissue weight and plasma and CSF volume, respectively.

Naloxon Quantification. Naloxone serum levels were determined using LC-MS/MS after alkaline extraction with ethyl acetate on an API 5500 mass spectrometer (AB Sciex) with ES Interface in the positive MRM mode. Separation was performed on a pentafluorophenyl column 50 × 4.6 mm (MonoChrom MS) with acetonitrile and 1% acetic acid as mobile phase (40:60). Mass transitions for naloxone are 328.2–310.1 *m/z* and 328.2–212.0 *m/z*, respectively. Internal Standard was tizanidine with mass transition of 253.8–44.0 *m/z*.

Statistical Analysis. Statistical analysis was carried out using SPSS 16.0 (SPSS Inc.). All data are reported as means ± SEM. Differences between groups were detected with Student's two-tailed *t* test for comparing two groups and two-factorial analysis of variance, followed by Fischer's LSD post hoc analysis for more than two groups. Significance was evaluated at a probability of 5% or less (<0.05).

ACKNOWLEDGMENTS. We thank Claudia Schwitter and Raissa Lerner for technical assistance with the eCBs extraction. We also gratefully acknowledge the technical assistance of Christof Dormann. This study was supported by funding from the Deutsche Forschungsgemeinschaft (SFB636-B3 to P.G. and FOR926 to B.L.).

1. Boecker H, et al. (2008) The runner's high: Opioidergic mechanisms in the human brain. *Cereb Cortex* 18(11):2523–2531.
2. Raichlen DA, Foster AD, Gerdeman GL, Seillier A, Giuffrida A (2012) Wired to run: Exercise-induced endocannabinoid signaling in humans and cursorial mammals with implications for the 'runner's high'. *J Exp Biol* 215(Pt 8):1331–1336.
3. Fuss J, Gass P (2010) Endocannabinoids and voluntary activity in mice: Runner's high and long-term consequences in emotional behaviors. *Exp Neurol* 224(1):103–105.
4. Carr DB, et al. (1981) Physical conditioning facilitates the exercise-induced secretion of beta-endorphin and beta-lipotropin in women. *N Engl J Med* 305(10):560–563.
5. Sparling PB, Giuffrida A, Piomelli D, Rosskopf L, Dietrich A (2003) Exercise activates the endocannabinoid system. *Neuroreport* 14(17):2209–2211.
6. Hill MN, et al. (2010) Endogenous cannabinoid signaling is required for voluntary exercise-induced enhancement of progenitor cell proliferation in the hippocampus. *Hippocampus* 20(4):513–523.
7. Galdino G, et al. (2014) The endocannabinoid system mediates aerobic exercise-induced antinociception in rats. *Neuropharmacology* 77:313–324.
8. Dubreucq S, et al. (2013) Ventral tegmental area cannabinoid type-1 receptors control voluntary exercise performance. *Biol Psychiatry* 73(9):895–903.
9. van Praag H, Christie BR, Sejnowski TJ, Gage FH (1999) Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA* 96(23): 13427–13431.
10. van Praag H, Kempermann G, Gage FH (1999) Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 2(3):266–270.
11. Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA (2011) Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. *Nature* 476(7361):458–461.
12. Massa F, et al. (2010) Alterations in the hippocampal endocannabinoid system in diet-induced obese mice. *J Neurosci* 30(18):6273–6281.
13. Monory K, et al. (2006) The endocannabinoid system controls key epileptogenic circuits in the hippocampus. *Neuron* 51(4):455–466.
14. Fuss J, et al. (2013) Hippocampal serotonin-1A receptor function in a mouse model of anxiety induced by long-term voluntary wheel running. *Synapse* 67(10):648–655.
15. Fuss J, et al. (2010) Voluntary exercise induces anxiety-like behavior in adult C57BL/6J mice correlating with hippocampal neurogenesis. *Hippocampus* 20(3):364–376.
16. Cluny NL, et al. (2010) A novel peripherally restricted cannabinoid receptor antagonist, AM6545, reduces food intake and body weight, but does not cause malaise, in rodents. *Br J Pharmacol* 161(3):629–642.
17. Gatley SJ, Gifford AN, Volkow ND, Lan R, Makriyannis A (1996) 123I-labeled AM251: A radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. *Eur J Pharmacol* 307(3):331–338.
18. McLaughlin PJ, et al. (2003) The cannabinoid CB1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats. *Behav Pharmacol* 14(8):583–588.
19. Lomazzo E, et al. (2015) Therapeutic potential of inhibitors of endocannabinoid degradation for the treatment of stress-related hyperalgesia in an animal model of chronic pain. *Neuropsychopharmacology* 40(2):488–501.
20. Glass PS, Jhaveri RM, Smith LR (1994) Comparison of potency and duration of action of nalmefene and naloxone. *Anesth Analg* 78(3):536–541.