Aus dem Johannes-Müller-Centrum für Physiologie,
Institut für Neurophysiologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Neurotrophinerge Wirkung auf die postnatale Umkehr der GABA-Wirkung

Zur Erlangung des akademischen Grades
Doctor medicinae (Dr. med.)
vorgelegt der Medizinischen Fakultät Charité
- Universitätsmedizin Berlin

von

Jan Akyeli
aus Essen

Gutachter: 1.: Prof. Dr. med. R. Grantyn

2.: Prof. Dr. med. B. W. Böttiger

3.: Prof. Dr. rer. nat. E. Günther

Datum der Promotion: 22. 6. 2007

Inhaltsverzeichnis

5. Zusammenfassung

Die schnelle postsynaptische Hemmung durch GABA führt zumeist zu Chlorideinstrom durch den anionenselektiven $GABA_A$-Kanal und dadurch zu Hyperpolarisation. Hyperpolarisation tritt jedoch nur auf, wenn die intrazelluläre Chloridkonzentration durch den neuronalen K-Cl-Cotransporter KCC2 unter das Gleichgewichtspotential erniedrigt wird, so dass eine Triebkraft für Chlorideinstrom besteht. Da dieser Transporter aber erst im Laufe der Entwicklung auftritt, bewirkt GABA bei Säugern zunächst noch eine Depolarisation. Dieser depolarisierenden GABA Wirkung wird eine wichtige Rolle in der Synaptogenese zugeschrieben.

In der vorliegenden Arbeit wurde der Einfluss des chronischen Fehlens des Neurotrophins BDNF auf die entwicklungsabhängige Regulation von KCC2 untersucht, die bisher kontrovers diskutiert wurde. Neuronale $GABA_A$-Rezeptor-Aktivität wurde in akuten Schnittpräparaten der spätembryonalen und früh postnatalen SGS des *Colliculus superior* charakterisiert und in Präparationen von Wildtyp- (*bdnf+/+*) und BDNF-defizienten (*bdnf-/-*) Mäusen verglichen. Ganzzell– oder Gramicidin–peforierte Patch-Ableitungen und Kalziumfluoreszenz-Messungen wurden durchgeführt, um Membranpotentiale, Umkehrpotentiale der GABA-induzierten Ströme($E_{(GABA)}$), beziehungsweise GABA-induzierte Kalziumsignale zu messen. Exogen appliziertes GABA war in der Lage, Neurone des Wildtyps bis zum postnatalen Tag (P) 1 zu depolarisieren. Zum Zeitpunkt P2 zeigten *bdnf*+/+- und *bdnf-/-*-Neurone einen signifkanten Unterschied in $E_{(GABA)}$, mit positiveren Werten in *bdnf-/-*-Mäusen. Das chronische Fehlen von BDNF verzögerte den Verlust der depolarisierenden GABA-Wirkung. Zwischen P1 und P3 zeigten die GABA-induzierten Kalziumfluoreszenzsignale in *bdnf-/-*-Mäusen höhere Amplituden. In jedem getesteten Alter (von P0 bis P8), fehlte den Neuronen des Wildtyps mRNA des Cl^- Transporters NKCC1, wogegen mRNA für KCC2 immer vorhanden war. Es zeigte sich, dass der Verlust der depolarisierenden GABA-Wirkung zum Zeitpunkt P2 in der *bdnf+/+*-Präparation mit einem Anstieg der KCC2-Immunoreaktivität in der Plasmamembran assoziiert war. Im Gegensatz dazu zeigten *bdnf-/-*-Neurone ein mehr diffuses KCC2-Verteilungsmuster über das gesamte Cytosol. Als Schlussfolgerung beschleunigt das Vorhandensein von BDNF das Verschwinden der depolarisierenden GABA-Wirkung über eine Stimulation der KCC2-

Insertion in die neuronale Plasmamembran. Das Fehlen von BDNF verlängert somit das Zeitfenster, in dem GABA depolarisierend wirkt.

A. Danksagung

Mein besonderer Dank gilt Frau Prof. Dr. Rosemarie Grantyn für die freundliche Überlassung des Themas. Ihre kontinuierliche und ausgezeichnete Betreuung haben die Fertigstellung dieser Arbeit ermöglicht.

Herrn Dr. Christian Henneberger danke ich für seine konstruktiven Hinweise, die ein wesentlicher Anstoss in einer wichtigen Etappe dieser Arbeit waren und für die kritische Durchsicht dieses Manuskripts.

Ich danke Dr. Sergej Kirischuk und Dr. Jochen Meier für die Einarbeitung in die Methoden der Elektrophysiologie, der Fluoreszenzmessung und der Molekularbiologie. Für die Hilfsbereitschaft bei der Tierzucht und der Genotypisierung danke ich Frau Kerstin Rückwardt.

B. Literaturverzeichnis

1. F. Aguado, M. A. Carmona, E. Pozas, et al. BDNF regulates spontaneous correlated activity at early developmental stages by increasing synaptogenesis and expression of the K+/Cl- co-transporter KCC2. *Development*, 2003;130(7):1267-1280.

2. H. Bading, D. D. Ginty, und M. E. Greenberg. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. *Science*, 1993;260(5105):181-186.

3. V. Balakrishnan, M. Becker, S. Lohrke, H. G. Nothwang, E. Guresir, und E. Friauf. Expression and function of chloride transporters during development of inhibitory neurotransmission in the auditory brainstem. *J.Neurosci.*, 2003;23(10):4134-4145.

4. Z. C. Baquet, P. C. Bickford, und K. R. Jones. Brain-derived neurotrophic factor is required for the establishment of the proper number of dopaminergic neurons in the substantia nigra pars compacta. *J.Neurosci.*, 2005;25(26):6251-6259.

5. M. Barbacid. The Trk family of neurotrophin receptors. *J.Neurobiol.*, 1994; 25(11):1386-1403.

6. M. Barbacid. Structural and functional properties of the TRK family of neurotrophin receptors. *Ann.N.Y.Acad.Sci.*, 1995;766442-458.

7. P. Bartho, J. A. Payne, T. F. Freund, und L. Acsady. Differential distribution of the KCl cotransporter KCC2 in thalamic relay and reticular nuclei. *Eur.J.Neurosci.*, 2004;20(4):965-975.

8. M. S. Beattie, A. W. Harrington, R. Lee, et al. ProNGF induces p75-mediated death of oligodendrocytes following spinal cord injury. *Neuron*, 2002;36(3):375-386.

9. M. Becker, H. G. Nothwang, und E. Friauf. Differential expression pattern of chloride transporters NCC, NKCC2, KCC1, KCC3, KCC4, and AE3 in the developing rat auditory brainstem. *Cell Tissue Res.*, 2003;312(2):155-165.

10. Y. Ben Ari, R. Khazipov, X. Leinekugel, O. Caillard, und J. L. Gaiarsa. GABAA, NMDA and AMPA receptors: a developmentally regulated 'menage a trois'. *Trends Neurosci.*, 1997;20(11):523-529.

11. Y. Ben Ari und G. L. Holmes. The multiple facets of gamma-aminobutyric acid dysfunction in epilepsy. *Curr.Opin.Neurol.*, 2005;18(2):141-145.

12. U. Bergman, F. W. Rosa, C. Baum, B. E. Wiholm, und G. A. Faich. Effects of exposure to benzodiazepine during fetal life. *Lancet*, 1992;340(8821):694-696.

13. D. K. Binder, M. J. Routbort, und J. O. McNamara. Immunohistochemical evidence of seizure-induced activation of trk receptors in the mossy fiber pathway of adult rat hippocampus. *J.Neurosci.*, 1999;19(11):4616-4626.

14. K. E. Binns. The synaptic pharmacology underlying sensory processing in the superior colliculus. *Prog.Neurobiol.*, 1999;59(2):129-159.

15. F. E. Bloom und L. L. Iversen. Localizing 3H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature*, 1971;229(5287):628-630.

16. R. Blum, K. W. Kafitz, und A. Konnerth. Neurotrophin-evoked depolarization requires the sodium channel Na(V)1.9. *Nature*, 2002;419(6908):687-693.

17. J. Bormann, O. P. Hamill, und B. Sakmann. Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. *J.Physiol*, 1987;385243-286.

18. I. Brunig, S. Penschuck, B. Berninger, J. Benson, und J. M. Fritschy. BDNF reduces miniature inhibitory postsynaptic currents by rapid downregulation of GABA(A) receptor surface expression. *Eur.J.Neurosci.*, 2001;13(7):1320-1328.

19. R. F. Bulleit und T. Hsieh. MEK inhibitors block BDNF-dependent and -independent expression of GABA(A) receptor subunit mRNAs in cultured mouse cerebellar granule neurons. *Brain Res.Dev.Brain Res.*, 2000;119(1):1-10.

20. M. Caleo, E. Menna, S. Chierzi, M. C. Cenni, und L. Maffei. Brain-derived neurotrophic factor is an anterograde survival factor in the rat visual system. *Curr.Biol.*, 2000;10(19):1155-1161.

21. M. Canossa, O. Griesbeck, B. Berninger, G. Campana, R. Kolbeck, und H. Thoenen. Neurotrophin release by neurotrophins: implications for activity-dependent neuronal plasticity. *Proc.Natl.Acad.Sci.U.S.A*, 1997;94(24):13279-13286.

22. M. V. Chao und B. L. Hempstead. p75 and Trk: a two-receptor system. *Trends Neurosci.*, 1995;18(7):321-326.

23. E. Cherubini, J. L. Gaiarsa, und Y. Ben Ari. GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.*, 1991;14(12):515-519.

24. E. Cherubini und F. Conti. Generating diversity at GABAergic synapses. *Trends Neurosci.*, 2001;24(3):155-162.

25. I. Choi, C. Aalkjaer, E. L. Boulpaep, und W. F. Boron. An electroneutral sodium/bicarbonate cotransporter NBCn1 and associated sodium channel. *Nature*, 2000;405(6786):571-575.

26. I. Chudotvorova, A. Ivanov, S. Rama, et al. Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. *J.Physiol*, 2005;566(Pt 3):671-9.

27. S. E. Clark, M. Garret, und B. Platt. Postnatal alterations of GABA receptor profiles in the rat superior colliculus. *Neuroscience*, 2001;104(2):441-454.

28. F. Crestani, M. Lorez, K. Baer, et al. Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat.Neurosci.*, 1999;2(9):833-839.

29. F. Crestani, J. R. Martin, H. Mohler, und U. Rudolph. Mechanism of action of the hypnotic zolpidem in vivo. *Br.J.Pharmacol.*, 2000;131(7):1251-1254.

30. S. J. Czuczwar und P. N. Patsalos. The new generation of GABA enhancers. Potential in the treatment of epilepsy. *CNS.Drugs*, 2001;15(5):339-350.

31. K. P. Das, S. L. Chao, L. D. White, et al. Differential patterns of nerve growth factor, brain-derived neurotrophic factor and neurotrophin-3 mRNA and protein levels in developing regions of rat brain. *Neuroscience*, 2001;103(3):739-761.

32. A. M. Davies, K. F. Lee, und R. Jaenisch. p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. *Neuron*, 1993;11(4):565-574.

33. P. Davies, B. Anderton, J. Kirsch, A. Konnerth, R. Nitsch, und M. Sheetz. First one in, last one out: the role of gabaergic transmission in generation and degeneration. *Prog.Neurobiol.*, 1998;55(6):651-658.

34. M. D. Edwards, A. M. White, und B. Platt. Characterisation of rat superficial superior colliculus neurones: firing properties and sensitivity to GABA. *Neuroscience*, 2002;110(1):93-104.

35. J. Eilers, T. D. Plant, N. Marandi, und A. Konnerth. GABA-mediated Ca2+ signalling in developing rat cerebellar Purkinje neurones. *J.Physiol*, 2001;536(Pt 2):429-437.

36. H. Einat, P. Yuan, T. D. Gould, et al. The role of the extracellular signal-regulated kinase signaling pathway in mood modulation. *J.Neurosci.*, 2003;23(19):7311-7316.

37. R. Enz, J. H. Brandstatter, E. Hartveit, H. Wassle, und J. Bormann. Expression of GABA receptor rho 1 and rho 2 subunits in the retina and brain of the rat. *Eur.J.Neurosci.*, 1995;7(7):1495-1501.

38. P. Ernfors, K. F. Lee, und R. Jaenisch. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature*, 1994;368(6467):147-150.

39. M. Fiore, G. Dell'Omo, E. Alleva, und H. P. Lipp. A comparison of behavioural effects of prenatally administered oxazepam in mice exposed to open-fields in the laboratory and the real world. *Psychopharmacology (Berl)*, 1995;122(1):72-77.

40. M. Frerking, R. C. Malenka, und R. A. Nicoll. Brain-derived neurotrophic factor (BDNF) modulates inhibitory, but not excitatory, transmission in the CA1 region of the hippocampus. *J.Neurophysiol.*, 1998;80(6):3383-3386.

41. T. F. Freund und G. Buzsaki. Interneurons of the hippocampus. *Hippocampus*, 1996;6(4):347-470.

42. J. M. Fritschy, C. Schweizer, I. Brunig, und B. Luscher. Pre- and post-synaptic mechanisms regulating the clustering of type A gamma-aminobutyric acid receptors (GABAA receptors). *Biochem.Soc.Trans.*, 2003;31(Pt 4):889-892.

43. D. O. Frost, Y. T. Ma, T. Hsieh, M. E. Forbes, und J. E. Johnson. Developmental changes in BDNF protein levels in the hamster retina and superior colliculus. *J.Neurobiol.*, 2001;49(3):173-187.

44. S. Furukawa, Y. Sugihara, F. Iwasaki, et al. Brain-derived neurotrophic factor-like immunoreactivity in the adult rat central nervous system predominantly distributed in neurons with substantial amounts of brain-derived neurotrophic factor messenger RNA or responsiveness to brain-derived neurotrophic factor. *Neuroscience*, 1998;82(3):653-670.

45. K. Ganguly, A. F. Schinder, S. T. Wong, und M. Poo. GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell*, 2001;105(4):521-532.

46. L. R. Gauthier, B. C. Charrin, M. Borrell-Pages, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell*, 2004;118(1):127-138.

47. J. Goggi, I. A. Pullar, S. L. Carney, und H. F. Bradford. Modulation of neurotransmitter release induced by brain-derived neurotrophic factor in rat brain striatal slices in vitro. *Brain Res.*, 2002;941(1-2):34-42.

48. L. J. Goodman, J. Valverde, F. Lim, et al. Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. *Mol.Cell Neurosci.*, 1996;7(3):222-238.

49. R. Grantyn, R. Jüttner, und J. Meier. Development and use-dependent modification of synaptic connections in the visual layers of the rodent superior colliculus. *In W.C.Hall und A.Moschovakis (eds.) The superior colliculus. CRC Press, Boca Raton*, 2004;173-210.

50. L. A. Greene und D. R. Kaplan. Early events in neurotrophin signalling via Trk and p75 receptors. *Curr.Opin.Neurobiol.*, 1995;5(5):579-587.

51. O. Griesbeck, M. Canossa, G. Campana, et al. Are there differences between the secretion characteristics of NGF and BDNF? Implications for the modulatory role of neurotrophins in activity-dependent neuronal plasticity. *Microsc.Res.Tech.*, 1999;45(4-5):262-275.

52. G. Grynkiewicz, M. Poenie, und R. Y. Tsien. A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J.Biol.Chem.*, 1985;260(6):3440-3450.

53. A. Gulacsi, C. R. Lee, A. Sik, et al. Cell type-specific differences in chloride-regulatory mechanisms and GABA(A) receptor-mediated inhibition in rat substantia nigra. *J.Neurosci.*, 2003;23(23):8237-8246.

54. A. Haapasalo, I. Sipola, K. Larsson, et al. Regulation of TRKB surface expression by brain-derived neurotrophic factor and truncated TRKB isoforms. *J.Biol.Chem.*, 2002;277(45):43160-43167.

55. A. R. Harvey. Expression of low affinity NGF (p75) receptors in rat superior colliculus: studies in vivo, in vitro, and in fetal tectal grafts. *Exp.Neurol.*, 1994;130(2):237-249.

56. K. Hashimoto, E. Shimizu, und M. Iyo. Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res.Brain Res.Rev.*, 2004;45(2):104-114.

57. W. Haubensak, F. Narz, R. Heumann, und V. Lessmann. BDNF-GFP containing secretory granules are localized in the vicinity of synaptic junctions of cultured cortical neurons. *J.Cell Sci.*, 1998;111 (Pt 11)1483-1493.

58. C. Henneberger, R. Grantyn, und T. Rothe. Rapid genotyping of newborn gene mutant mice. *J.Neurosci.Methods*, 2000;100(1-2):123-126.

59. C. Henneberger, R. Juttner, T. Rothe, und R. Grantyn. Postsynaptic action of BDNF on GABAergic synaptic transmission in the superficial layers of the mouse superior colliculus. *J.Neurophysiol.*, 2002;88(2):595-603.

60. C. Henneberger, S. Kirischuk S, und R. Grantyn. Brain-derived neurotrophic factor modulates GABAergic synaptic transmission by enhancing presynaptic glutamic acid decarboxylase 65 levels, promoting asynchronous release

and reducing the number of activated postsynaptic receptors. *Neuroscience*, 2005;135(3):749-63.

61. R. M. Holsinger, J. Schnarr, P. Henry, V. T. Castelo, und M. Fahnestock. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. *Brain Res.Mol.Brain Res.*, 2000;76(2):347-354.

62. E. J. Huang und L. F. Reichardt. Neurotrophins: roles in neuronal development and function. *Annu.Rev.Neurosci.*, 2001;24677-736.

63. Z. J. Huang, A. Kirkwood, T. Pizzorusso, et al. BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell*, 1999;98(6):739-755.

64. C. A. Hübner, D. E. Lorke, und I. Hermans-Borgmeyer. Expression of the Na-K-2Cl-cotransporter NKCC1 during mouse development. *Mech.Dev.*, 2001a;102(1-2):267-269.

65. C. A. Hübner, V. Stein, I. Hermans-Borgmeyer, T. Meyer, K. Ballanyi, und T. J. Jentsch. Disruption of KCC2 reveals an essential role of K-Cl cotransport already in early synaptic inhibition. *Neuron*, 2001b;30(2):515-524.

66. T. Isa und Y. Saito. The direct visuo-motor pathway in mammalian superior colliculus; novel perspective on the interlaminar connection. *Neurosci.Res.*, 2001;41(2):107-113.

67. L. Jasmin, S. D. Rabkin, A. Granato, A. Boudah, und P. T. Ohara. Analgesia and hyperalgesia from GABA-mediated modulation of the cerebral cortex. *Nature*, 2003;424(6946):316-320.

68. R. Jüttner, J. Meier, und R. Grantyn. Slow IPSC kinetics, low levels of alpha1 subunit expression and paired-pulse depression are distinct properties of

neonatal inhibitory GABAergic synaptic connections in the mouse superior colliculus. *Eur.J.Neurosci.*, 2001;13(11):2088-2098.

69. K. W. Kafitz, C. R. Rose, H. Thoenen, und A. Konnerth. Neurotrophin-evoked rapid excitation through TrkB receptors. *Nature*, 1999;401(6756):918-921.

70. Y. Kakazu, N. Akaike, S. Komiyama, und J. Nabekura. Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons. *J.Neurosci.*, 1999;19(8):2843-2851.

71. M. R. Kaplan, D. B. Mount, und E. Delpire. Molecular mechanisms of NaCl cotransport. *Annu.Rev.Physiol*, 1996;58649-668.

72. D. L. Kaufman, C. R. Houser, und A. J. Tobin. Two forms of the gamma-aminobutyric acid synthetic enzyme glutamate decarboxylase have distinct intraneuronal distributions and cofactor interactions. *J.Neurochem.*, 1991;56(2):720-723.

73. C. K. Kellogg, J. Yao, und G. L. Pleger. Sex-specific effects of in utero manipulation of GABA(A) receptors on pre- and postnatal expression of BDNF in rats. *Brain Res.Dev.Brain Res.*, 2000;121(2):157-167.

74. W. Kelsch, S. Hormuzdi, E. Straube, A. Lewen, H. Monyer, und U. Misgeld. Insulin-like growth factor 1 and a cytosolic tyrosine kinase activate chloride outward transport during maturation of hippocampal neurons. *J.Neurosci.*, 2001;21(21):8339-8347.

75. S. Kirischuk, J. Akyeli, R. Iosub, und R. Grantyn. Pre- and postsynaptic contribution of GABAC receptors to GABAergic synaptic transmission in rat collicular slices and cultures. *Eur.J.Neurosci.*, 2003;18(4):752-758.

76. S. Kirischuk, R. Jüttner, und R. Grantyn. Time-matched pre- and postsynaptic changes of GABAergic synaptic transmission in the developing mouse superior colliculus. *J.Physiol*, 2005;563(Pt 3):795-807.

77. J. Kirsch und H. Betz. Glycine-receptor activation is required for receptor clustering in spinal neurons. *Nature* , 1998;392(6677):717-720.

78. S. Kobayashi, C. W. Morgans, J. R. Casey, und R. R. Kopito. AE3 anion exchanger isoforms in the vertebrate retina: developmental regulation and differential expression in neurons and glia. *J.Neurosci.*,1994;14(10):6266-6279.

79. K. Kohara, A. Kitamura, M. Morishima, und T. Tsumoto. Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science*, 2001;291(5512):2419-2423.

80. M. Kojima, N. Takei, T. Numakawa, et al. Biological characterization and optical imaging of brain-derived neurotrophic factor-green fluorescent protein suggest an activity-dependent local release of brain-derived neurotrophic factor in neurites of cultured hippocampal neurons. *J.Neurosci.Res.*, 2001;64(1):1-10.

81. M. Korte, P. Carroll, E. Wolf, G. Brem, H. Thoenen, und T. Bonhoeffer. Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc.Natl.Acad.Sci.U S A*, 1995;92(19):8856-8860.

82. K. Kraszewski und R. Grantyn. Unitary, quantal and miniature GABA-activated synaptic chloride currents in cultured neurons from the rat superior colliculus. *Neuroscience*, 1992;47(3):555-570.

83. K. Kuriyama, M. Hirouchi, und H. Kimura. Neurochemical and molecular pharmacological aspects of the GABA(B) receptor. *Neurochem.Res.*, 2000;25(9-10):1233-1239.

84. A. Kyrozis und D. B. Reichling. Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. *J.Neurosci.Methods*, 1995;57(1):27-35.

85. L. Laegreid, G. Hagberg, und A. Lundberg. Neurodevelopment in late infancy after prenatal exposure to benzodiazepines--a prospective study. *Neuropediatrics*, 1992;23(2):60-67.

86. T. Lang, I. Wacker, J. Steyer, et al. Ca2+-triggered peptide secretion in single cells imaged with green fluorescent protein and evanescent-wave microscopy. *Neuron*, 1997;18(6):857-863.

87. H. Lee, C. X. Chen, Y. J. Liu, E. Aizenman, und K. Kandler. KCC2 expression in immature rat cortical neurons is sufficient to switch the polarity of GABA responses. *Eur.J.Neurosci.*, 2005;21(9):2593-2599.

88. R. Lee, P. Kermani, K. K. Teng, und B. L. Hempstead. Regulation of cell survival by secreted proneurotrophins. *Science*, 2001;294(5548):1945-1948.

89. X. Leinekugel, I. Khalilov, H. McLean, et al. GABA is the principal fast-acting excitatory transmitter in the neonatal brain. *Adv.Neurol.*, 1999;79189-201.

90. R. Levi-Montalcini, H. Meyer, und V. Hamburger. In vitro experiments on the effects of mouse sarcomas 180 and 37 on the spinal and sympathetic ganglia of the chick embryo. *Cancer Res.*, 1954;14(1):49-57.

91. M. Levivier, S. Przedborski, C. Bencsics, und U. J. Kang. Intrastriatal implantation of fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevents degeneration of dopaminergic neurons in a rat model of Parkinson's disease. *J.Neurosci.*, 1995;15(12):7810-7820.

92. H. Li, J. Tornberg, K. Kaila, M. S. Airaksinen, und C. Rivera. Patterns of cation-chloride cotransporter expression during embryonic rodent CNS development. *Eur.J.Neurosci.*, 2002;16(12):2358-2370.

93. Y. X. Li, Y. Xu, D. Ju, H. A. Lester, N. Davidson, und E. M. Schuman. Expression of a dominant negative TrkB receptor, T1, reveals a requirement for

presynaptic signaling in BDNF-induced synaptic potentiation in cultured hippocampal neurons. *Proc.Natl.Acad.Sci.U.S.A*, 1998;95(18):10884-10889.

94. B. Lom, J. Cogen, A. L. Sanchez, T. Vu, und S. Cohen-Cory. Local and target-derived brain-derived neurotrophic factor exert opposing effects on the dendritic arborization of retinal ganglion cells in vivo. *J.Neurosci.*, 2002;22(17):7639-7649.

95. J. J. LoTurco, D. F. Owens, M. J. Heath, M. B. Davis, und A. R. Kriegstein. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis.*Neuron*, 1995;15(6):1287-1298.

96. B. Lu und A. Chow. Neurotrophins and hippocampal synaptic transmission and plasticity. *J.Neurosci.Res.*, 1999;58(1):76-87.

97. B. Lu und W. Gottschalk. Modulation of hippocampal synaptic transmission and plasticity by neurotrophins.*Prog.Brain Res.*, 2000;128231-241.

98. J. Lu, M. Karadsheh, und E. Delpire. Developmental regulation of the neuronal-specific isoform of K-Cl cotransporter KCC2 in postnatal rat brains. *J.Neurobiol.*, 1999;39(4):558-568.

99. A. Ludwig, H. Li, M. Saarma, K. Kaila, und C. Rivera. Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. *Eur.J.Neurosci.*, 2003;18(12):3199-3206.

100. P. D. Lukasiewicz, E. D. Eggers, B. T. Sagdullaev, und M. A. McCall. GABAC receptor-mediated inhibition in the retina.*Vision Res.*, 2004;44(28):3289-3296.

101. R. D. Lund und J. S. Lund. Development of synaptic patterns in the superior colliculus of the rat.*Brain Res.*, 1972;42(1):1-20.

102. F. H. Marshall, K. A. Jones, K. Kaupmann, und B. Bettler. GABAB receptors - the first 7TM heterodimers. *Trends Pharmacol.Sci.*, 1999;20(10):396-399.

103. S. Marty, B. Berninger, P. Carroll, und H. Thoenen. GABAergic stimulation regulates the phenotype of hippocampal interneurons through the regulation of brain-derived neurotrophic factor. *Neuron*, 1996;16(3):565-570.

104. K. Matsui, J. Hasegawa, und M. Tachibana. Modulation of excitatory synaptic transmission by GABA(C) receptor-mediated feedback in the mouse inner retina. *J.Neurophysiol.*, 2001;86(5):2285-2298.

105. G. Matthews, G. S. Ayoub, und R. Heidelberger. Presynaptic inhibition by GABA is mediated via two distinct GABA receptors with novel pharmacology. *J.Neurosci.*, 1994;14(3 Pt 1):1079-1090.

106. A. K. McAllister. Subplate neurons: a missing link among neurotrophins, activity, and ocular dominance plasticity? *Proc.Natl.Acad.Sci.U.S.A*, 1999;96(24):13600-13602.

107. M. A. McCall, P. D. Lukasiewicz, R. G. Gregg, und N. S. Peachey. Elimination of the rho1 subunit abolishes GABA(C) receptor expression and alters visual processing in the mouse retina. *J.Neurosci.*, 2002;22(10):4163-4174.

108. B. M. McLean, A. J. Pittman, und D. C. Lo. Brain-derived neurotrophic factor differentially regulates excitatory and inhibitory synaptic transmission in hippocampal cultures. *J.Neurosci.*, 2000;20(9):3221-3232.

109. A. K. Mehta und M. K. Ticku. An update on GABAA receptors. *Brain Res.Brain Res.Rev.*, 1999;29(2-3):196-217.

110. J. Meier, J. Akyeli, S. Kirischuk, und R. Grantyn. GABA(A) receptor activity and PKC control inhibitory synaptogenesis in CNS tissue slices. *Mol.Cell Neurosci.*, 2003;23(4):600-613.

111. J. P. Merlio, P. Ernfors, M. Jaber, und H. Persson. Molecular cloning of rat trkC and distribution of cells expressing messenger RNAs for members of the trk family in the rat central nervous system. *Neuroscience*, 1992;51(3):513-532.

112. J. P. Merlio, P. Ernfors, Z. Kokaia, et al. Increased production of the TrkB protein tyrosine kinase receptor after brain insults. *Neuron*, 1993;10(2):151-164.

113. R. Miles. Neurobiology. A homeostatic switch. *Nature*, 1999;397(6716):215-216.

114. R. R. Mize. Immunocytochemical localization of gamma-aminobutyric acid (GABA) in the cat superior colliculus. *J.Comp.Neurol.*, 1988;276(2):169-187.

115. R. R. Mize. The organization of GABAergic neurons in the mammalian superior colliculus. *Prog.Brain Res.*, 1992;90219-248.

116. Y. Mizoguchi, H. Ishibashi, und J. Nabekura. The action of BDNF on GABAA currents changes from potentiating to suppressing during maturation of rat hippocampal CA1 pyramidal neurons. *J.Physiol.*, 2003;548(Pt 3):703-709.

117. I. Mody. Aspects of the homeostaic plasticity of GABAA receptor-mediated inhibition. *J.Physiol*, 2005;562(Pt 1):37-46.

118. D. D. Murphy, N. B. Cole, und M. Segal. Brain-derived neurotrophic factor mediates estradiol-induced dendritic spine formation in hippocampal neurons. *Proc.Natl.Acad.Sci.U.S.A*, 1998;95(19):11412-11417.

119. M. Nishikawa, M. Hirouchi, und K. Kuriyama. Functional coupling of Gi subtype with GABAB receptor/adenylyl cyclase system: analysis using a reconstituted system with purified GTP-binding protein from bovine cerebral cortex. *Neurochem.Int.*, 1997;31(1):21-25.

120. A. Nykjaer, R. Lee, K. K. Teng, et al. Sortilin is essential for proNGF-induced neuronal cell death. *Nature*, 2004;27(6977):843-848.

121. K. Obrietan, X. B. Gao, und A. N. Van Den Pol. Excitatory actions of GABA increase BDNF expression via a MAPK-CREB-dependent mechanism--a positive

feedback circuit in developing neurons. *J. Neurophysiol., 2002.Aug.;88.(2.):1005.-15.*,88(2):1005-1015.

122. Y. Okada. The distribution and function of gamma-aminobutyric acid (GABA) in the superior colliculus. *Prog.Brain Res.*, 1992;90249-262.

123. H. G. Olbrich und H. Braak. Ratio of pyramidal cells versus non-pyramidal cells in sector CA1 of the human Ammon's horn. *Anat.Embryol.(Berl)*, 1985;173(1):105-110.

124. R. W. Olsen, T. M. DeLorey, M. Gordey, und M. H. Kang. GABA receptor function and epilepsy. *Adv.Neurol.*, 1999;79:499-510.

125. D. F. Owens, L. H. Boyce, M. B. Davis, und A. R. Kriegstein. Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. *J.Neurosci.*, 1996;16(20):6414-6423.

126. K. Parain, M. G. Murer, Q. Yan, et al. Reduced expression of brain-derived neurotrophic factor protein in Parkinson's disease substantia nigra. *Neuroreport*, 1999;10(3):557-561.

127. J. Paul, K. Gottmann, und V. Lessmann. NT-3 regulates BDNF-induced modulation of synaptic transmission in cultured hippocampal neurons. *Neuroreport*, 2001;12(12):2635-2639.

128. J. A. Payne. Functional characterization of the neuronal-specific K-Cl cotransporter: implications for [K+]o regulation. *Am.J.Physiol*, 1997;273(5 Pt 1):C1516-C1525.

129. K. L. Perkins und R. K. Wong. Ionic basis of the postsynaptic depolarizing GABA response in hippocampal pyramidal cells. *J.Neurophysiol.*, 1996;76(6):3886-3894.

130. M. D. Plotkin, M. R. Kaplan, L. N. Peterson, S. R. Gullans, S. C. Hebert, und E. Delpire. Expression of the Na(+)-K(+)-2Cl- cotransporter BSC2 in the nervous system. *Am.J.Physiol*, 1997;272(1 Pt 1):C173-C183.

131. G. S. Pollock, E. Vernon, M. E. Forbes, et al. Effects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus, and cerebellum. *J.Neurosci.*, 2001;21(11):3923-3931.

132. J. S. Rhee, S. Ebihara, und N. Akaike. Gramicidin perforated patch-clamp technique reveals glycine-gated outward chloride current in dissociated nucleus solitarii neurons of the rat. *J.Neurophysiol.*, 1994;72(3):1103-1108.

133. C. Rivera, J. Voipio, J. A. Payne, et al. The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature*, 1999;397(6716):251-255.

134. C. Rivera, H. Li, J. Thomas-Crusells, et al. BDNF-induced TrkB activation down-regulates the K+-Cl- cotransporter KCC2 and impairs neuronal Cl- extrusion. *J.Cell Biol.*, 2002;159(5):747-752.

135. C. Rivera, J. Voipio, und K. Kaila. Two developmental switches in GABAergic signalling: the K+-Cl- cotransporter KCC2 and carbonic anhydrase CAVII. *J.Physiol*, 2005;562(Pt 1):27-36.

136. A. A. Roberts, G. L. Pleger, und C. K. Kellogg. Effect of prenatal exposure to diazepam on brain GABA(A) receptor mRNA levels in rats examined at late fetal or adult ages.*Dev.Neurosci.*, 2001;23(2):135-144.

137. J. Rohrbough und N. C. Spitzer. Regulation of intracellular Cl- levels by Na(+)-dependent Cl- cotransport distinguishes depolarizing from hyperpolarizing GABAA receptor-mediated responses in spinal neurons. *J.Neurosci.*, 1996;16(1):82-91.

138. T. Rothe, R. Bahring, P. Carroll, und R. Grantyn. Repetitive firing deficits and reduced sodium current density in retinal ganglion cells developing in the absence of BDNF. *J.Neurobiol.*, 1999;40(3):407-419.

139. R. Sala, A. Viegi, F. M. Rossi, et al. Nerve growth factor and brain-derived neurotrophic factor increase neurotransmitter release in the rat visual cortex.*Eur.J.Neurosci.*, 1998;10(6):2185-2191.

140. W. R. Schäbitz, C. Berger, R. Kollmar, et al. Effect of brain-derived neurotrophic factor treatment and forced arm use on functional motor recovery after small cortical ischemia. *Stroke*, 2004;35(4):992-997.

141. V. Schuler, C. Luscher, C. Blanchet, et al. Epilepsy, hyperalgesia, impaired memory, and loss of pre- and postsynaptic GABA(B) responses in mice lacking GABA(B(1)). Neuron, 2001;31(1):47-58.

142. E. M. Schuman. Neurotrophin regulation of synaptic transmission. Curr.Opin.Neurobiol., 1999;9(1):105-109.

143. B. Singh, C.Henneberger, D. Betances et al. Altered balance of glutamatergic/GABAergic synaptic input and associated changes in dendrite morphology after BDNF expression in BDNF-deficient hippocampal neurons. *J Neurosci.,* 2006 Jul 5;26(27):7189-200.

144. J. J. Soghomonian und D. L. Martin. Two isoforms of glutamate decarboxylase: why? *Trends Pharmacol.Sci.*, 1998;19(12):500-505.

145. V. Stein, I. Hermans-Borgmeyer, T. J. Jentsch, und C. A. Hubner. Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. *J.Comp Neurol.*, 2004;468(1):57-64.

146. K. Strange, T. D. Singer, R. Morrison, und E. Delpire. Dependence of KCC2 K-Cl cotransporter activity on a conserved carboxy terminus tyrosine residue. *Am.J.Physiol Cell Physiol*, 2000;279(3):C860-C867.

147. D. Sun und S. G. Murali. Na+-K+-2Cl- cotransporter in immature cortical neurons: A role in intracellular Cl- regulation. *J.Neurophysiol.*, 1999;81(4):1939-1948.

148. M. K. Sun, T. J. Nelson, H. Xu, und D. L. Alkon. Calexcitin transformation of GABAergic synapses: from excitation filter to amplifier. *Proc.Natl.Acad.Sci.U.S.A*, 1999;96(12):7023-7028.

149. K. W. Sung, M. Kirby, M. P. McDonald, D. M. Lovinger, und E. Delpire. Abnormal GABAA receptor-mediated currents in dorsal root ganglion neurons isolated from Na-K-2Cl cotransporter null mice. *J.Neurosci.*, 2000;20(20):7531-7538.

150. C. T. Supuran, A. Scozzafava, und A. Casini. Carbonic anhydrase inhibitors. *Med.Res.Rev.*, 2003;23(2):146-189.

151. H. K. Teng, K. K. Teng, R. Lee, et al. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J.Neurosci.*, 2005;25(22):5455-5463.

152. H. Thoenen. Neurotrophins and neuronal plasticity. *Science*, 1995;270(5236):593-598.

153. C. L. Thompson, M. H. Tehrani, E. M. J. Barnes, und F. A. Stephenson. Decreased expression of GABAA receptor alpha6 and beta3 subunits in stargazer mutant mice: a possible role for brain-derived neurotrophic factor in the regulation of cerebellar GABAA receptor expression? *Brain Res.Mol.Brain Res.*, 1998;60(2):282-290.

154. S. Titz, M. Hans, W. Kelsch, A. Lewen, D. Swandulla, und U. Misgeld. Hyperpolarizing inhibition develops without trophic support by GABA in cultured rat midbrain neurons. *J.Physiol*, 2003;550(Pt 3):719-730.

155. F. W. Turek und S. Losee-Olson. A benzodiazepine used in the treatment of insomnia phase-shifts the mammalian circadian clock. *Nature*, 1986;321(6066):167-168.

156. M. H. Tuszynski, U. HS, J. Alksne, et al. Growth factor gene therapy for Alzheimer disease. *Neurosurg.Focus.*, 2002;13(5):e5.

157. N. Upton. Mechanisms of action of new antiepileptic drugs: rational design and serendipitous findings. *Trends Pharmacol.Sci.*, 1994;15(12):456-463.

158. C. Vale, J. Schoorlemmer, und D. H. Sanes. Deafness disrupts chloride transporter function and inhibitory synaptic transmission. *J.Neurosci.*, 2003;23(20):7516-7524.

159. C. Vicario-Abejon, C. Collin, R. D. McKay, und M. Segal. Neurotrophins induce formation of functional excitatory and inhibitory synapses between cultured hippocampal neurons. *J.Neurosci.*, 1998;18(18):7256-7271.

160. M. L. Vizuete, J. L. Venero, C. Vargas, M. Revuelta, A. Machado, und J. Cano. Potential role of endogenous brain-derived neurotrophic factor in long-term neuronal reorganization of the superior colliculus after bilateral visual deprivation. *Neurobiol.Dis.*, 2001;8(5):866-880.

161. H. Wang, Y. Yan, D. B. Kintner, C. Lytle, und D. Sun. GABA-mediated trophic effect on oligodendrocytes requires Na-K-2Cl cotransport activity. *J.Neurophysiol.*, 2003;90(2):1257-1265.

162. J. Wang, D. B. Reichling, A. Kyrozis, und A. B. MacDermott. Developmental loss of GABA- and glycine-induced depolarization and Ca2+ transients in embryonic rat dorsal horn neurons in culture. *Eur.J.Neurosci.*, 1994;6(8):1275-1280.

163. J. Wang, S. Liu, U. Haditsch, et al. Interaction of calcineurin and type-A GABA receptor gamma 2 subunits produces long-term depression at CA1 inhibitory synapses. *J.Neurosci.*, 2003;23(3):826-836.

164. S. S. Warton, M. Perouansky, und R. Grantyn. Development of GABAergic synaptic connections in vivo and in cultures from the rat superior colliculus. *Brain Res.Dev.Brain Res.*, 1990;52(1-2):95-111.

165. G. Weskamp und L. F. Reichardt. Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. *Neuron*, 1991;6(4):649-663.

166. C. Wetmore, Y. H. Cao, R. F. Pettersson, und L. Olson. Brain-derived neurotrophic factor: subcellular compartmentalization and interneuronal transfer as visualized with anti-peptide antibodies. *Proc.Natl.Acad.Sci.U.S.A*, 1991;88(21):9843-9847.

167. J. R. Williams, J. W. Sharp, V. G. Kumari, M. Wilson, und J. A. Payne. The neuron-specific K-Cl cotransporter, KCC2. Antibody development and initial characterization of the protein. *J.Biol.Chem.*, 1999;274(18):12656-12664.

168. W. Wisden, D. J. Laurie, H. Monyer, und P. H. Seeburg. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J.Neurosci.*, 1992 ;12(3):1040-1062.

169. C. G. Wong, T. Bottiglieri, und O. C. Snead. GABA, gamma-hydroxybutyric acid, and neurological disease. *Ann.Neurol.*, 2003;54 Suppl 6:S3-12.S3-12.

170. N. H. Woo, H. K. Teng, C. J. Siao, et al. Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat.Neurosci.*, 2005;8(8):1069-1077.

171. W. L. Wu, L. Ziskind-Conhaim, und M. A. Sweet. Early development of glycine- and GABA-mediated synapses in rat spinal cord. *J.Neurosci.*, 1992;12(10):3935-3945.

172. Q. Xu, E. de la Cruz, und S. A. Anderson. Cortical interneuron fate determination: diverse sources for distinct subtypes? *Cereb.Cortex.*, 2003 ;13(6):670-676.

173. J. Yamada, A. Okabe, H. Toyoda, W. Kilb, H. J. Luhmann, und A. Fukuda. Cl- uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1. *J.Physiol*, 2004 ;557(Pt 3):829-841.

174. Q. Yan, M. J. Radeke, C. R. Matheson, J. Talvenheimo, A. A. Welcher, und S. C. Feinstein. Immunocytochemical localization of TrkB in the central nervous system of the adult rat. *J.Comp.Neurol.*, 1997;378(1):135-157.

175. L. Zhang, I. Spigelman, und P. L. Carlen. Development of GABA-mediated, chloride-dependent inhibition in CA1 pyramidal neurones of immature rat hippocampal slices. *J.Physiol*, 1991;44425-49.

176. L. Zhu, D. Lovinger, und E. Delpire. Cortical neurons lacking KCC2 expression show impaired regulation of intracellular chloride. *J.Neurophysiol.*, 2005;93(3):1557-1568.

C. Eigene Veröffentlichungen

Beiträge in wissenschaftlichen Zeitschriften:

J. Meier, J. Akyeli, S. Kirischuk und R. Grantyn. GABA(A) receptor activity and PKC control inhibitory synaptogenesis in CNS tissue slices. *Mol Cell Neurosci.,* 2003 Aug;23(4):600-13.

S. Kirischuk, J. Akyeli, R. Iosub und R. Grantyn. Pre- and postsynaptic contribution of GABAC receptors to GABAergic synaptic transmission in rat collicular slices and cultures. *Eur J Neurosci.,* 2003 Aug;18(4):752-8.

Abstrakta:

Akyeli J., Henneberger C., Meier J., Rothe T. und Grantyn R.
Presynaptic effects of BDNF on GABAergic synaptic transmission in the postnatal mouse superior colliculus (SC). *FENS Abstr. vol.1*, 2002; A206.1.

Meier J, Akyeli J, Kirischuk S und Grantyn R. GABAA Receptor Activity and PKC Control Inhibitory Synaptogenesis in CNS Tissue Slices. *Synaptogenesis Wien*, 2003

Akyeli J, Meier J, Kirischuk S, Grantyn R. BDNF regulates the KCC2-dependent switch from depolarizing to hyperpolarizing GABA action in the mouse superior colliculus. *IBRO,* 2003; Abstr.2019.

D. Abkürzungsverzeichnis

ACh	Acetylcholin
ACSF	künstlicher Liquor (artificial cerebrospinal fluid)
AMPA	(S)-®-Amino-3-Hydroxy-5-Methyl-4-isoxazolepropionsäure
BDNF	brain derived neurotrophic factor
$[Ca^{2+}]_I$	intrazelluläre Kalziumkonzentration
$[Cl^-]_I$	intrazelluläre Chloridkonzentration
CS	*Colliculus superior*
DAG	Diacylglycerol
$E_{(GABA)}$	Gleichgewichtspotential GABA-induzierter Ströme
eIPSC	evozierter inhibitorischer postsynaptischer Strom
GABA	γ-Aminobuttersäure
GABAR	GABA-Rezeptor
GAD	Glutamat-Decarboxylase
IGF	Insulin-like growth factor
IP_3	Inositoltriphosphat
IPSC	inhibitorischer postsynaptischer Strom
KCC	Kalium-Chlorid-Kotransporter
LTP	Langzeitpotenzierung
MAPK	mitogen-aktivierte Proteinkinase
mIPSC	Miniatur-IPSC
NKCC	Natrium-Kalium-Chlorid-Kotransporter
NMDA	N-Methyl-D-Aspartat
NT	Neurotrophin
PCR	Polymerasekettenreaktion

PKC	Proteinkinase C
PLC	Phospholipase C
PNS	peripheres Nervensystem
$p75^{NTR}$	P75-Neurotrophinrezeptor
RT-PCR	reverse Transkriptase Polymerasekettenreaktion
RGZ	retinale Ganglienzelle
SGS	*Stratum griseum superficiale*
sIPSC	spontaner inhibitorischer postsynaptischer Strom
SOp	*Stratum opticum*
TNF	Tumornekrosefaktor
TrkA,TrkB,TrkC	Tyrosinkinase-A,-B und -C
VACC	spannungsaktivierte Kalziumkanäle
ZNS	zentrales Nervensystem

E. Lebenslauf

„Mein Lebenslauf wird aus Datenschutzgründen in der elektronischen Version meiner Arbeit nicht mit veröffentlicht.“

F. Selbständigkeitserklärung

„Ich, Jan Akyeli, erkläre, dass ich die vorgelegte Dissertationsschrift mit demThema: Neurotrophinerge Wirkung auf die postnatale Umkehr der GABA-Wirkung, selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Berlin, den 2.9.2006