J Neural Transm (2000) 107: 133–143

Journal of Neural Transmission

Adult treatment with methamphetamine transiently decreases dentate granule cell proliferation in the gerbil hippocampus

G. Teuchert-Noodt, **R. R. Dawirs**, and **K. Hildebrandt**

Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Bielefeld, Federal Republic of Germany

Received January 8, 1999; accepted July 12, 1999

Summary. The objective of the present study was to examine whether acute treatment with the recreational drug methamphetamine influences adult granule cell proliferation in the dentate gyrus of the hippocampus. For that purpose, at the age of postnatal day 90 adult male gerbils (Meriones unguiculatus) received a single dose of either methamphetamine (25 mg/kg; i.p.) or saline. Proliferation of granule cells was identified by in-vivo labeling with 5-bromo-2'-desoxyuridine (BrdU) which was applied either simultaneously with methamphetamine or 36 h after administration of the drug. BrdU-labeled granule cell nuclei were identified in consecutive horizontal slices along the mid-septotemporal axis of the hippocampus and light-microscopically quantified 7 days after the BrdU-labeling. It was found that in both saline- and methamphetamine-treated animals there was a highly significant spatial septotemporal gradient in granule cell proliferation with numbers of BrdU-labeled cells gradually declining from the septal towards the temporal pole. The acute treatment with methamphetamine suppressed granule cell proliferation by about 28% and the septotemporal gradient of mitotic activity became significantly attenuated. It was further found that 36 h after the drug challenge granule cell proliferation rates had been restored almost to the control values along the whole septotemporal axis of the hippocampus. The present results are discussed with regard to (1) pharmacological regulation of neurogenesis in the hippocampus and (2) probable clues they may provide for both understanding the biological correlates of psychotic disorders and evolution of future concepts in neuropharmacological intervention.

Keywords: Cell proliferation, dentate gyrus, methamphetamine, psychosis.

Introduction

There is now ample evidence that neurogenesis occurs in the adult hippocampus of mammals including non-human primates and man. In the subgranular zone of the adult dentate gyrus multipotent progenitor cells continue to

provide newly born granule cells throughout life until old age (Kuhn et al., 1996; Kempermann et al., 1998; Gage et al., 1998; Eriksson et al., 1998). The functional significance of this ongoing cell proliferation is as yet obscure. Nevertheless, we have enough reason to assume that adult neurogenesis in the hippocampus might be part of an adaptive strategy, continuously reorganizing limbo-prefrontal networks in the brain (cf. Dawirs et al., 1992; Hildebrandt et al., 1998). In this respect, it is of particular interest to learn more about extrinsic (environmental) and intrinsic (biochemical) signals regulating granule cell proliferation in vivo. A growing set of data indicates that adult neurogenesis is under complex control of neurotransmitters, hormones and certain growth factors (for recent review see: Gage et al., 1998; Cameron et al., 1998). We have recently shown that acute doses of haloperidol significantly augmented granule cell proliferation, and we have discussed the probable role of dopamine in the in-vivo regulation of neurogenesis in the adult hippocampus (Dawirs et al., 1998). In this connection, it has been reported that dopamine agonists have antiproliferative effects in non-neural systems (Carretero et al., 1996; Morikawa et al., 1994). The present study was conducted to evaluate whether methamphetamine, which has been shown to increase concentrations of extracellular dopamine in the brain (Bennet et al., 1998), has comparable antiproliferative activity in the adult hippocampus. For that purpose, we administered a single dose of methamphetamine to adult gerbils. We examined neurogenesis of granule cells using in-vivo labeling of dividing cells with 5-bromo-2'-desoxyuridine (BrdU) (1) acutely with the drug challenge and (2) 36 h after application of the drug. Labeled cell nuclei were light-microscopically detected and quantified after a single dose of either methamphetamine or saline.

Material and methods

Animals and pharmacological treatment

Male gerbils (Meriones unguiculatus) were bred in the laboratory in standard cages measuring 55 × 33 × 20 cm (Makrolon® Cage type IV). At the age of postnatal day 30, the animals were weaned and subsequently kept singly in standard home cages measuring 27 × 22 × 15 cm (Makrolon® Cage type III). Food and water were provided ad libitum. All gerbils were on natural day/night cycles. At the age of postnatal day 90, 20 gerbils received a single dose of methamphetamine (25 mg/kg; i.p.). For that purpose, appropriate amounts of methamphetamine hydrochloride (Sigma) were diluted in 0.5 ml saline immediately before injection. Ten control animals received equivalent volumes of saline. Simultaneously, 10 methamphetamine-treated animals and the controls were BrdU-labeled. The other 10 methamphetamine-treated animals were BrdU-labeled 36 h after application of the drug.

BrdU-Labeling

Proliferation of granule cells was identified by in-vivo labeling with the thymidine analog 5-bromo-2'-desoxyuridine (BrdU). BrdU is incorporated into DNA of dividing cells during the S-phase and is detected using a specific monoclonal antibody (Gratzner, 1982). A single dose of BrdU (30 mg/kg, i.p.) (Cell Proliferation Kit RPN 20, Amersham, Braunschweig, Germany) was injected 7 days prior to sacrifice. The animals were sacrificed by overdose with ether. The brain was immediately dissected and frozen (−20°C).

After freezing, the whole brain was serially cut in 12-μm thick horizontal sections on a Frigocut (−12°C) (Reichert-Jung, Model 2700) and slices were mounted on slides, dried in air and fixed with acetone. The following incubations were performed at room temperature with gentle agitation. The sections were washed in 0.1 M sodium phosphate buffer (PBS, pH 7.4, three 10-min washes) and acid denatured in 2 N HCl for 15 min. Sections were then neutralized and washed in PBS (three 10-min washes). To eliminate endogenous peroxidase activity tissue was incubated in 2% H_2O_2 (dissolved in methanol) for 20 min. Sections were then washed in PBS (three times for 10 min). To avoid any unspecific reactions of the first and second antibody with the tissue the slices were incubated with 10% normal goat serum (NGS) (Sigma, Deisenhofen, Germany) for 20 min in a sealed, hydrated container. Sections were then washed in PBS (three 10-min washes). Following this the slices were incubated in anti-BrdU first antibody (Kit, Amersham; anti-BrdU/nuclease, 1:100 in aqua dest.) for 75 min. After washing with PBS (three times for 10 min) the slices were incubated with the second antibody (Amersham; peroxidase anti-mouse IgG2a, 1.5:100 in dilution buffer) for 45 min. Sections were washed three times in PBS and subsequently treated with diaminobenzidine (DAB, 0.025%; Amersham) and a reagent containing a substrate and intensifier for 3 min to reveal the peroxidase label. After washing in aqua dest. The slices were slightly counterstained with Richardson, dehydrated, mounted in Eukitt (Riedel-de Haën, Seelze, Germany) and coverslipped. Immunochemical controls were run for each specimen, with the primary antibody being replaced by PBS in control sections. No labeling was observed in controls.

Quantification and data analysis

Every 6th consecutive section was selected from the mid-septotemporal level and a total of 38 slices was evaluated in each animal. BrdU-labeled nuclei were identified in the subgranular and granular layer of the dentate gyrus and manually counted by light microscopy (300-fold magnification). Results are given as the mean number of BrdU-labeled cell nuclei in either the left or right dentate gyrus of each slice. Mean values were computed as arithmetic means ± S.D., and compared by t-test (two-tailed) with preceding F-test. Data were analyzed with bivariante analysis of variance (ANOVA) with repeated measurements (Sachs, 1974).

Results

A representative sample of BrdU-labeled cell nuclei in the dentate gyrus of adult gerbils at the age of 90 days which had either received methamphetamine or saline is shown in Fig. 1. Mean individual numbers ± standard deviation of BrdU-labeled cell nuclei in each of the 38 consecutive slices along the mid-septotemporal axis are given for saline- and methamphetamine-treated animals (Fig. 2). Single data comprise all detectable labeled cell nuclei in the subgranular and granular layer of the left and right dentate gyrus of standard 12-μm slices. Total mean individual numbers ± standard deviation of BrdU-labeled cell nuclei in each of 10 animals are given as deviation from the mean in the left and right dentate gyrus of saline- and methamphetamine-treated animals (Fig. 3A–F). The data reveal that effects of methamphetamine on mitotic activity in the dentate gyrus are not lateralized (Fig. 3G). About 28% less BrdU-labeled cell nuclei could be detected along the entire mid-septotemporal axis acutely after the methamphetamine challenge ($p < 0.001$) (Fig. 3H). Within 36 h after methamphetamine treatment the numbers of detectable BrdU-labeled cell nuclei had been restored to control values (Fig. 3H).

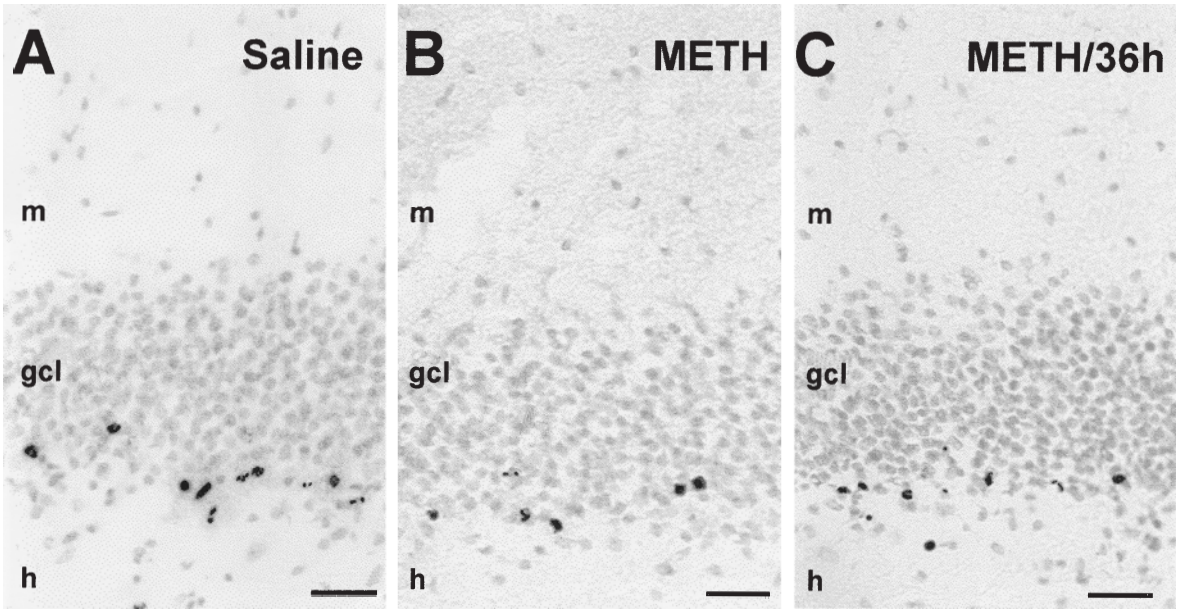

Fig. 1. Brightfield photomicrographs of BrdU-labeled cell nuclei in the dentate gyrus of saline- (**A**) and methamphetamine-treated animals which received a single pulse of BrdU either simultaneously with (**B**) or 36 h after the drug (**C**); granule cell layer (gcl); hilus (h), molecular layer (m); scale 50 μm (**A**, **B**, **C**)

In the left and right dentate gyrus of both saline- and methamphetamine-treated animals numbers of BrdU-labeled cell nuclei continuously declined from the septal towards the temporal pole of the hippocampus. ANOVA revealed this septotemporal gradient to be highly significant in each pharmacological group. ANOVA further revealed that mitotic activity was not lateralized along the whole septotemporal gradient (Table 1). Therefore, data derived from both hemispheres have been pooled in each pharmacological group (Fig. 4). The methamphetamine treatment had an acute and significant influence on BrdU-labeling along the septotemporal axis (row effect), and alters the interrelationship between numbers of BrdU-labeled cell nuclei and slice number (interaction) (Fig. 4A, Table 1). Within 36 h after the single drug challenge the septotemporal gradient had been restored almost to control values (Fig. 4B, C, Table 1). Regression analysis of saline- ($S = 33.2 - 0.5x$; $r = 0.89$; $p = 2.5 \times 10^{-28}$) and methamphetamine-treated animals analyzed for BrdU-incorporation either acutely ($M = 25.2 - 0.4x$; $r = 0.88$; $p = 1.1 \times 10^{-26}$) or 36 h after application of the drug ($M/36 = 32.3 - 0.6x$; $r = 0.92$; $p = 9.1 \times 10^{-30}$) reveals that the rate of decline of BrdU-labeled cell nuclei along the septotemporal axis ($\Delta_s = -0.54$; $p = 4.3 \times 10^{-14}$) was attenuated by about 20% after methamphetamine treatment ($\Delta_M = -0.43$; $p = 2.9 \times 10^{-13}$). 36 h after the drug challenge this rate of decline had returned almost to control values ($\Delta_{M/36} = -0.58$; $p = 1.5 \times 10^{-16}$).

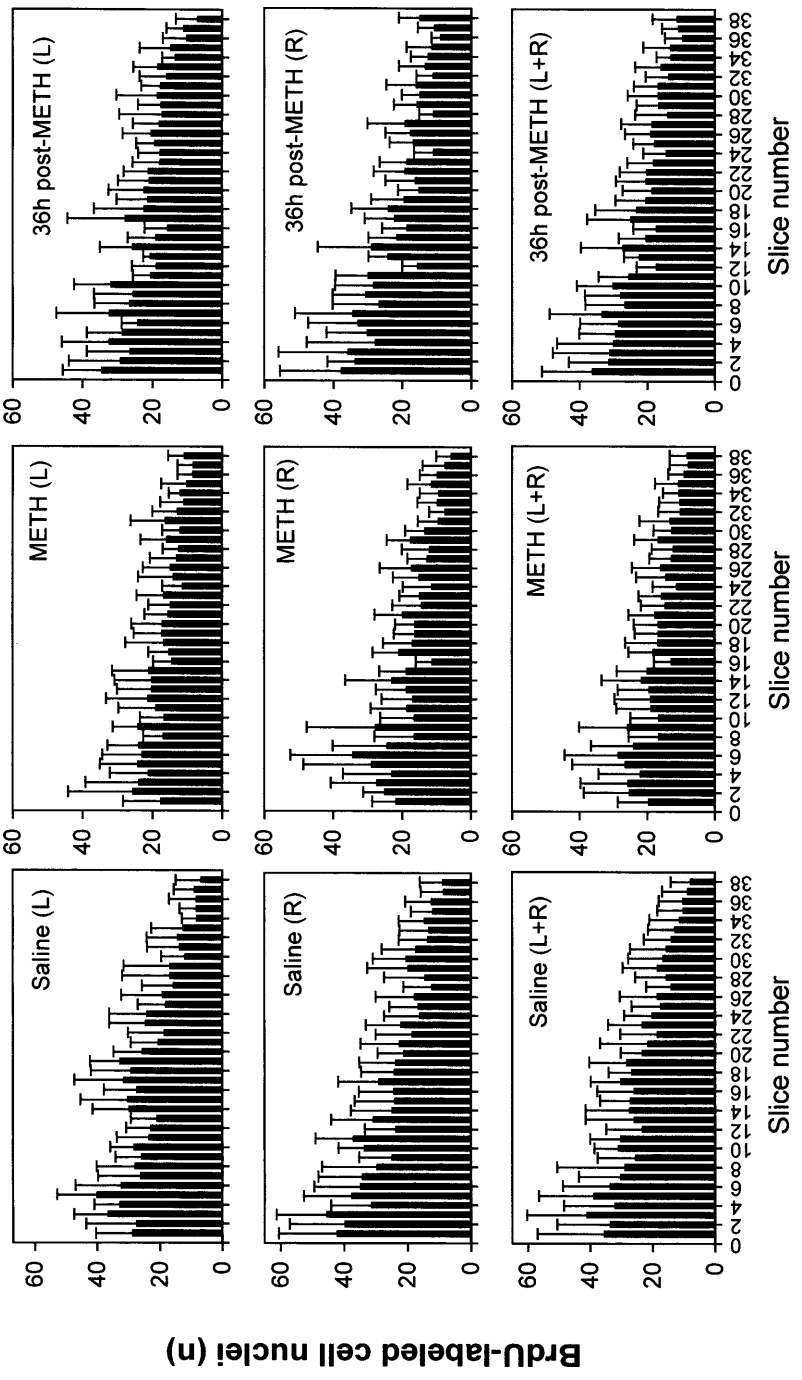

Fig. 2. Numbers of BrdU-labeled cell nuclei in the dentate gyrus of saline- and methamphetamine-treated animal which received a single pulse of BrdU either simultaneously with (METH) or 36 h after the drug (36 h post-METH); mean number ± standard deviation is given in each of 38 consecutive slices from the septal (slice number 1) to the temporal pole (slice number 38); mean numbers ± standard deviation were calculated from separate counts in the left (L) and right (R) dentate gyrus (n = 10)

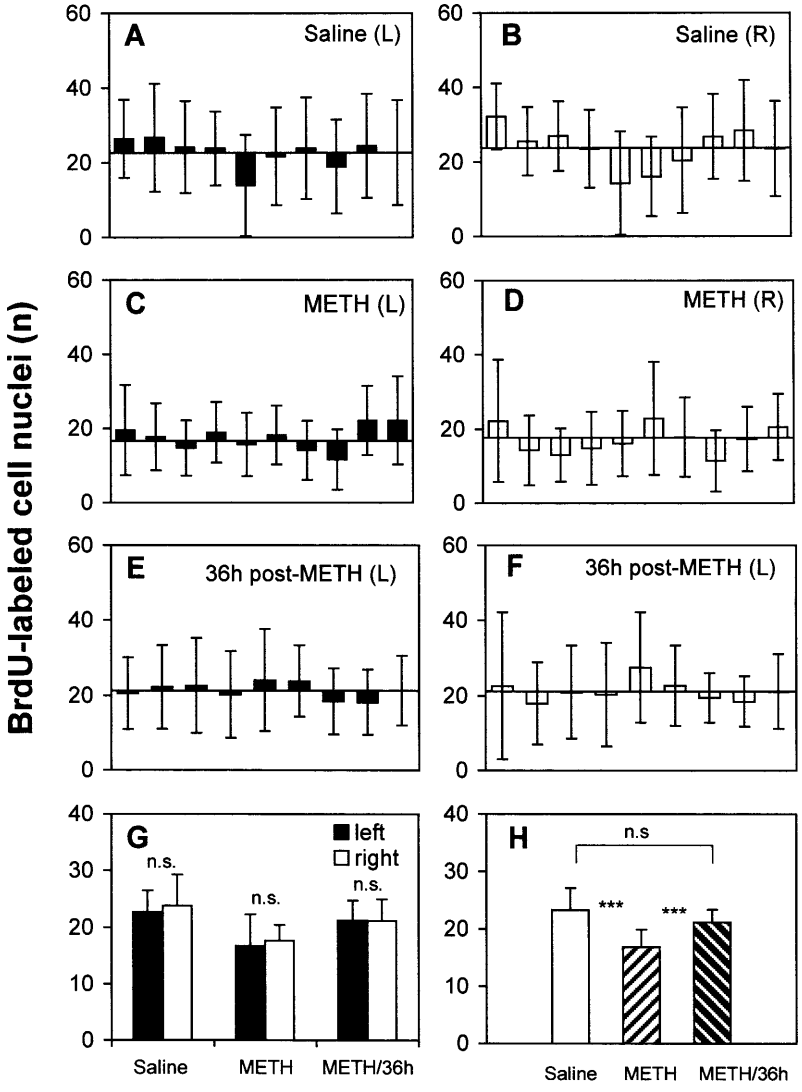

Fig. 3. A–F Total mean number of BrdU-labeled cell nuclei in the left (L) and right (R) dentate gyrus of saline- and methamphetamine-treated animal which received a single pulse of BrdU either simultaneously with (METH) or 36 h after the drug (36 h post-METH) (n = 38); mean number ± standard deviation is given as deviation from mean in each of 10 animals; **G** overall mean individual numbers of BrdU-labeled cell nuclei in the left and right dentate gyrus of each pharmacological group (n = 10); **H** overall mean individual numbers of BrdU-labeled cell nuclei comprising counts in both hemispheres (n = 20); methamphetamine (METH); 36 h post-METH (METH/36 h); not significant (n.s.); $p < 0.001$ (***)

Discussion

In the present study we investigated whether a single dose of methamphetamine influences granule cell neurogenesis in the hippocampus of adult gerbils. For that purpose we counted mitotic cells after in-vivo BrdU-labeling. We found that a single dose of methamphetamine acutely attenuated the mean individual proliferation rate in the subgranular zone by about 28%.

Table 1. Methamphetamine-induced alterations in granule cell proliferation along the septo-temporal axis; ANOVA analysis of laterality and drug treatment for septotemporal gradients in numbers of BrdU-labeled cell nuclei in the dentate gyrus; number of labeled cells acutely after methamphetamine treatment (METH) and 36 h after the drug (36 h post-METH)

ANOVA	Laterality (p-values)		
Reason of variance	left/right (Saline)	left/right (METH)	left/right (36 h post-METH)
Laterality (row effect)	n.s.	n.s.	n.s.
Septo-temporal gradient (column effect)	3.26×10^{-41}	7.29×10^{-30}	1.93×10^{-53}
Interaction	n.s.	n.s.	n.s.
ANOVA	Pharmacological treatment (p-values)		
Reason of variance	Saline/METH	METH/36 h post-METH	Saline/36 h post-METH
Pharmacological treatment (row effect)	4.03×10^{-16}	1.50×10^{-16}	0.01
Septo-temporal gradient (column effect)	1.92×10^{-48}	1.01×10^{-67}	2.89×10^{-56}
Interaction	0.004	0.028	0.007

Additionally, we found that drug-induced suppression of granule cell proliferation was only transient. 36 h after the single drug challenge mean individual proliferation rates in the methamphetamine-treated animals no longer differed from saline-treated controls. Further, we found a highly significant spatial gradient of cell proliferation in the dentate gyrus of adult gerbils with gradually decreasing mitotic activities along the septotemporal axis. In acute response to the methamphetamine treatment this septotemporal gradient became transiently diminished, and was restored to almost control values within 36 h after the drug challenge.

We have recently shown that acute doses of haloperidol significantly stimulate granule cell proliferation in the dentate gyrus adult gerbils (Dawirs et al., 1998). This is in accordance with mitogenic activities of haloperidol in non-neuronal tissues (Liu and Wolfe, 1996; Wegner et al., 1997). On the other hand several investigations indicate that dopamine and dopamine receptor agonists are capable of significantly suppressing cell proliferation in various non-neuronal systems (Pawlikowski et al., 1983; Johansen et al., 1985; Morikawa et al., 1994; Carretero et al., 1996; for recent discussion see Wegner et al., 1997). This is in accordance with antimitogenic effects of methamphetamine on progenitor cells in the adult dentate gyrus of gerbils as we report in the present study. Methamphetamine exerts its agonistic effects on dopamin-

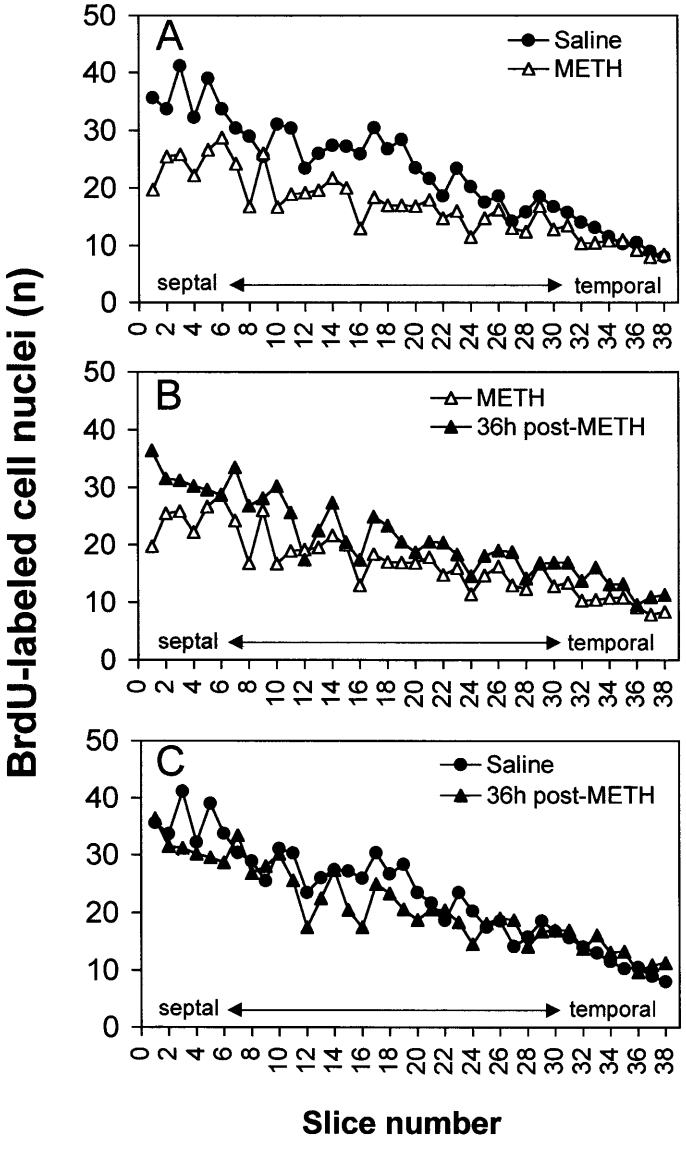

Fig. 4. Pooled numbers of BrdU-labeled cell nuclei in the dentate gyrus from both hemispheres gradually declining along the septotemporal axis; **A** methamphetamine (METH) treatment decreases the number of BrdU-labeled cell nuclei along the septotemporal axis and significantly weakens its spatial decline; **B**, **C** 36 h after application of the drug (36 h post-METH) numbers of BrdU-labeled cell nuclei were restored almost to control values from the septal to the temporal pole; results of ANOVA and regression analysis are given in Table 1 and in the text respectively

ergic functions mainly through stimulation of dopamine release. Our observation that methamphetamine-induced suppression of granule cell proliferation was reversible within 36 h tallies with findings that a single high dose of this drug causes rapid but transient inactivation of dopamine transporters which returned to control levels within 36 h (Hanson et al., 1998).

Our findings provide the first in-vivo model on psychoactive drugs either decreasing (haloperidol) or increasing (methamphetamine) dopamine functions in the brain, having inverse effects on neuronal cell proliferation in adult animals. Taking into account several ways of interaction between dopamine and other neurotransmitter systems we have argued that the dopaminergic system probably plays a pivotal role in controlling granule cell proliferation in the adult hippocampus (Dawirs et al., 1998; Hildebrandt et al., 1998). Other factors regulating mitogenic activities and neurogenesis in vivo have recently been discussed in detail elsewhere (Cameron et al., 1998; Gage et al., 1998).

We have shown that there is a highly significant spatial septotemporal gradient in granule cell proliferation with numbers of BrdU-labeled cell nuclei gradually declining from the septal towards the temporal pole of the hippocampus. This gradient is enhanced by acute doses of haloperidol (Dawirs et al., 1998) and is diminished by a single dose of methamphetamine (present study). Septal areas proved to be more sensitive than the more temporal areas to both the haloperidol and methamphetamine challenges. These spatially specific responses might be of particular interest in that they correlate with another septotemporal gradient in the hippocampus. It is well known that mesolimbic dopamine fibers innervate the hippocampus (Gasbarri et al., 1994), with more fibers innervating the subgranular proliferative zone in the temporal parts of the adult dentate gyrus than in the septal parts (Yoshida et al., 1988). Although the functional significance of this correlation is as yet obscure this may further support the view that mesolimbic dopamine activities might be significantly involved in regulation of granule cell neurogenesis in vivo.

From the present results we can conclude that haloperidol and methamphetamine both interfere with in-vivo mechanisms controlling neurogenesis in adult animals. Therefore, neuroactive drugs either decreasing or increasing dopaminergic functions in the brain might become promising candidates for selective manipulation of granule cell neurogenesis in the dentate gyrus in order to interfere with adaptive reorganization of limboprefrontal networks in schizophrenics (cf. recent discussion in Dawirs et al., 1998; Hildebrandt et al., 1998). In this respect several questions remain to be answered, for instance: (1) What are the effects of chronically applied psychoactive drugs on granule cell neurogenesis in adults and juveniles? (2) By which mechanisms might drug-induced alterations in adult neurogenesis be connected with drug-induced sprouting and synaptic reorganization in various areas of the brain (cf. Vincent et al., 1994; Eastwood et al., 1994; Nossoll et al., 1997; Dawirs et al., 1993, 1994, 1997)? (3) Besides proliferation, what are the effects of psychoactive drugs on granule cell differentation and survival?

Learning more about the morphogenetic properties of neuroactive drugs will provide the necessary basis for (1) a better understanding of functional and dysfunctional maturation of the brain and (2) evolution of pharmacological techniques which might be useful to selectively control these processes.

Acknowledgements

The authors are indebted to Mrs. U. Schroeder and Mr. F. Bagorda for technical assistance. Our sincere thanks are dedicated to Mrs. F. Misselbrook for correcting the English.

References

Bennet BA, Hollingsworth CK, Martin RS, Harp JJ (1998) Methamphetamine-induced alterations in dopamine transporter function. Brain Res 782: 219–227

Cameron HA, Hazel TG, McKay RDG (1998) Regulation of neurogenesis by growth factors and neurotransmitters. J Neurobiol 36: 287–306

Carretero J, Vazquez RJ, Santos M, Cacicedo L, Rubio M, Snchez-Franco F, Vazquez R (1996) Dopamine inhibits in-vitro release of VIP and proliferation of VIP-immunoreactive pituitary cells. Neuropeptides 30: 81–86

Dawirs RR, Teuchert-Noodt G, Kacza J (1992) Naturally occurring degrading events in axon terminals of the dentate gyrus and stratum lucidum in the spiny mouse (Acomys cahirinus) during maturation, adulthood and aging. Dev Neurosci 14: 210–220

Dawirs RR, Teuchert-Noodt G, Molthagen M (1993) Indication of methamphetamine-induced reactive synaptogenesis in the prefrontal cortex of gerbils (Meriones unguiculatus). Eur J Pharmacol 241: 89–97

Dawirs RR, Teuchert-Noodt G, Czaniera R (1994) The postnatal maturation of dopamine innervation in the prefrontal cortex of gerbils (Meriones unguiculatus) is sensitive to an early single dose of methamphetamine. A quantitative immunocytochemical study. J Brain Res 35: 195–204

Dawirs RR, Teuchert-Noodt G, Nossoll M (1997) Pharmacologically induced neural plasticity in the prefrontal cortex of adult gerbils (Meriones unguiculatus). Eur J Pharmacol 327: 117–123

Dawirs RR, Hildebrandt K, Teuchert-Noodt G (1998) Adult treatment with haloperidol increases dentate granule cell proliferation in the gerbil hippocampus. J Neural Transm 105: 317–327

Eastwood SL, Burnet PW, Harrison PJ (1994) Striatal synaptophysin expression and haloperidol-induced synaptic plasticity. Neuroreport 5: 677–680

Eriksson PS, Perfilieva E, Björk-Eriksson T, Alborn A -M, Nordborg C, Peterson DA, Gage FH (1998) Neurogenesis in the adult human hippocampus. Nature Med 4: 1313–1317

Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J (1998) Multipotent progenitor cells in the adult dentate gyrus. J Neurobiol 36: 249–266

Gasbarri A, Verney C, Innocenzi R, Campana E, Pacitti C (1994) Mesolimbic dopaminergic neurons innervating the hippocampal formation in the rat: a combined retrograde tracing and immunohistochemical study. Brain Res 668: 71–79

Gratzner HG (1982) Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science 218: 474–475

Hanson GR, Gibb JW, Metzger RR, Kokoshka JM, Fleckenstein AE (1998) Methamphetamine-induced rapid and reversible reduction in the activities of tryptophan hydroxylase and dopamine transporters: oxidative consequences? Ann NY Acad Sci 844: 103–107

Hildebrandt K, Teuchert-Noodt G, Dawirs RR (1998) A single neonatal dose of methamphetamine suppresses dentate granule cell proliferation in adult gerbils which is restored to control values by acute doses of haloperidol. J Neural Transm 106: 549–558

Johansen PW, Clausen OP, Haug E, Fossum S, Gautvik KM (1985) Effects of bromocriptine on cell cycle distribution and cell morphology in cultured rat pituitary adenoma cells. Acta Endocrinol 110: 319–328

Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18: 3206–3212
Kuhn HG, Dickinson-Anson H, and Gage FH (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16: 2027–2033
Liu Y, Wolfe Jr SA (1996) Haloperidol and spiperone potentiate murine splenic B cell proliferation. Immunopharmacol 34: 147–159
Morikawa K, Oseko F, Morikawa S (1994) Immunosuppressive activity of bromocriptine on human T lymphocyte function in vitro. Clin Exp Immunol 95: 514–518
Nossoll M, Teuchert-Noodt G, Dawirs RR (1997) A single dose of methamphetamine in neonatal gerbils affects adult prefrontal GABA innervation. Eur J Pharmacol 340: 3–5
Pawlikowski M, Jakubowski L, Kaluzewski B (1983) The antimitotic effect of bromocriptine on human fibroblasts. J Neural Transm 56: 91–95
Sachs L (1974) Angewandte Statistik. Springer, Berlin Heidelberg New York, p 545
Vincent SL, Adamee E, Sorensen I, Benes FM (1994) The effects of chronic haloperidol administration on GABA-immunoreactive axon terminals in rat medial prefrontal cortex. Synapse 17: 26–35
Wegner IC, Dawirs RR, Grond C, Teuchert-Noodt G (1997) Dopamine and the regulation of cell proliferation in gerbil (Meriones unguiculatus) pyloric mucosa. Life Sci 60: 2005–2011
Yoshida M, Sakai M, Kani K, Nagatsu I, Tanaka M (1988) The dopaminergic innervation as observed by immunohistochemistry using anti-dopamine serum in the rat cerebral cortex. Experientia 44: 700–702

Authors' address: Dr. R. R. Dawirs, Department of Neuroanatomy, Faculty of Biology, University of Bielefeld, Universitätsstrasse 25, D-33615 Bielefeld, Federal Republic of Germany, e-mail: R. Dawirs@biologie.uni-bielefeld.de