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# Modulation of hippocampal acetylcholine release after fimbria fornix



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**Abstract**

Female Long–Evans rats sustained electrolytic lesions of the fimbria and the dorsal fornix causing a partial lesion of the septohippocampal pathway. Two weeks later, the rats received intra-hippocampal grafts of fetal septal cell suspensions. Nine to twelve months later, the release of acetylcholine (ACh) in the hippocampus of sham-operated, lesion-only and grafted rats was measured by microdialysis. The extent of cholinergic (re)innervation was determined by acetylcholinesterase (AChE) staining and densitometry. In both lesion-only and grafted rats, the ratio of ACh release to AChE staining intensity was increased as compared to sham-operated rats, indicating a loss of endogenous inhibitory mechanisms. Scopolamine (0.5 mg/kg i.p.), a muscarinic antagonist, increased ACh release in all treatment groups. 8-OH-DPAT (0.5 mg/kg s.c.), an agonist at serotonergic $5HT_{1A}$-receptors, induced an increase of hippocampal ACh release in sham-operated rats. This effect was lost in lesion-only rats, but was fully restored by neuronal grafting. As 8-OH-DPAT influences hippocampal ACh release by a postsynaptic action, this finding indicates that the host brain exerts a serotonergic influence on the grafted cholinergic neurons. © 1997 Elsevier Science Ireland Ltd.

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The experimental lesion of the septohippocampal pathway is an animal model for central cholinergic dysfunction and Alzheimer's disease and has been used repeatedly to investigate functional effects of neuronal grafting. While grafted neurons may establish new synaptic contacts in the host brain and attenuate or even compensate for a variety of lesion-induced neurophysiological and behavioral dysfunctions [14], the question of whether grafted neurons show normal functional characteristics remains a great concern. Using the microdialysis technique, Nilsson et al. [11,12] have reported that basal forebrain grafts placed into the denervated hippocampus were able to release acetylcholine (ACh). An important finding was that this release is to some extent under the control of host afferents; sensory stimulation increased ACh release in sham-operated and grafted rats, but not in lesion-only rats, and the same observation was made after electrical stimulation of the habenula. Later it was reported that at least part of these host-derived regulations of grafted cholinergic neurons involved catecholaminergic fibres; thus, the ACh release in the grafted hippocampus was raised after administration of amphetamine and apomorphine [8]. In a recent experiment using brain slices [2], it was demonstrated that presynaptic mechanisms involving muscarinic autoreceptors and $5HT_{1B}$ heteroceptors were also functional on the axonal terminals of grafted cholinergic neurons, thereby inhibiting ACh release. In the present experiments, we have used the microdialysis technique to test (1) if the muscarinic autoregulation characterized in vitro [2] also operates in vivo and (2) if the grafted cholinergic neurons respond to the activation of $5HT_{1A}$ receptors with an increase of ACh release.

Twelve female Long–Evans rats (R. Janvier, France), 91 ± 1 days old, received an electrolytic lesion of the infracallosal component of the septo-hippocampal pathways (fimbria-fornix) as described previously [4]. This lesioning

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procedure produces a partial lesion of the hippocampus as shown by the loss of 50–70% of choline acetyltransferase (ChAT) activity [2,5]. The electrode was placed at five sites according to the following coordinates from Lambda: A +5.2 mm, L ±0.8 mm, V –3.3 mm; A +5.5 mm, L 0 mm, V –3.5 mm; A +5.8 mm, L ±1.8, V –3.8 mm. Sham-operated rats ($n = 6$) were subjected to scalp incision and removal of the bone overlying the dorsal parietal cortex. Two weeks after lesion surgery, a subgroup of lesioned rats ($n = 6$) received intra-hippocampal grafts of a cell suspension prepared from the septal region of Long–Evans fetuses (E 15) (for details, see [2]). The cell suspension was injected into the dorsal hippocampus at the following coordinates from Lambda: A +4.0 mm, L ±1.5 mm, V –3.0 mm; A +3.0 mm, L ±2.5 mm, V –3.3 mm (2 μl/site, 1 μl/min). Another subgroup consisted of lesioned rats which did not receive grafts ($n = 6$).

The in vivo release of ACh in the right dorsal hippocampus was examined by microdialysis in sham-operated, lesioned and grafted rats 9–12 months after transplantation. Self-made, I-shaped microdialysis probes (exchange length, 1 mm; 240 μm o.d.) were implanted into the dorsal hippocampus. The probe was positioned according to the coordinates from Lambda: A +2.5 mm, L –0.3 mm, V –3.8 mm and, therefore, was positioned in about 1 mm posterior to the most caudal graft. The experiments were carried out on conscious rats 1 or 2 days after probe implantation. The probe was perfused at 2 μl/min with artificial cerebrospinal fluid (CSF; 147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$ and 1.2 mM $MgCl_2$) containing 10 μM neostigmine, and ACh release was determined by high performance liquid chromatography (HPLC) as detailed previously [6]. On day 1, basal ACh release was determined; subsequently, 8-OH-DPAT (0.5 mg/kg) was given by s.c. injection, and ACh release was followed for 3–4 h (15 min samples). On day 2, the rats received scopolamine (0.5 mg/kg i.p.). Subsequently, the rats were transcardially perfused with phosphate-buffered paraformaldehyde, and the fixated brains were cut into coronal sections and stained for acetylcholinesterase (AChE) [5]. The intensity of the AChE staining was quantified in the right hippocampus using densitometry. For this purpose, the brown AChE stain was transformed into grey scaling using an EagleEye image analyzer, and the intensity of the grey levels was quantified with the NIH Image software using fixed frames to scan identical areas of the hippocampus. In addition, the sections were used to verify the correct location of the microdialysis probes, the survival of the grafts and the lesion extents.

Fig. 1 compares the AChE staining intensities and the basal ACh efflux observed in the three treatment groups. The lesioning procedure led to a highly significant reduction of AChE staining intensity in lesion-only rats which was fully restored by grafting. The grafts also restored the organotypic innervation pattern of the hippocampus as described previously (data not shown; cf. [5]). Additional densitometric analyses showed that the fimbria-fornix lesions did not affect the AChE-positivity in the neighboring cortical and thalamic areas (data not shown). It was remarkable that, after the partial lesioning procedure used in the present study, the basal release of ACh in lesion-only rats was only slightly and not significantly reduced ($P > 0.1$ vs. sham-operated rats; Fig. 1). As AChE staining and cholinergic innervation are closely correlated [10], the high ACh output in the partially lesioned hippocampus points to a hyperactivity of remaining cholinergic fibres, because the ratio of basal ACh release and AChE staining intensity was clearly increased in lesion-only rats. Thus, surviving cholinergic fibres in a partially damaged septo-hippocampal pathway maintained an increased basal transmitter output. It should be noted, however, that the same animals were unable to produce the high ACh output observed in sham-operated rats under stimulated conditions (Fig. 2).

Our microdialysis data indicate that the remaining cholinergic fibres in the hippocampus of fimbria-fornix-lesioned rats release significantly more ACh per time than those of sham-operated rats. This interpretation is in agreement with an earlier study of septo-hippocampal lesions in which partial fimbrial lesions reduced the hippocampal ChAT activity by 35% but did not result in a significant decrease of ACh turnover in the hippocampus [7]. Compensatory increases of ACh release have also been observed after aspirative lesions of the septo-hippocampal pathway [9], but these changes were measured 4 weeks after lesion surgery and were transient. We speculate that the lesion-induced hyperactivity of cholinergic fibers observed in the present study was most likely due to a lesion-induced destruction of (non-cholinergic) pathways which physiologically serve to modulate hippocampal ACh release in intact animals. Likely candidates for this modulatory function are GABAergic and serotonergic fibres which are known to be damaged by fimbria-fornix

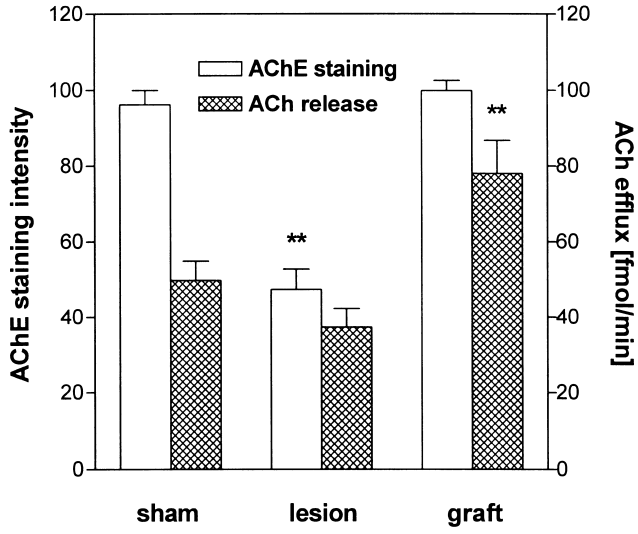


Fig. 1. AChE staining intensity (open bars) and basal ACh efflux (cross-hatched bars) in the hippocampi of sham-operated (sham), lesion-only (lesion) and grafted (graft) rats. Staining intensities were measured by densitometry and ACh efflux by HPLC as described in the text. Data are given as means ± SEM. $^{**}P < 0.01$ vs. sham (one-way ANOVA).

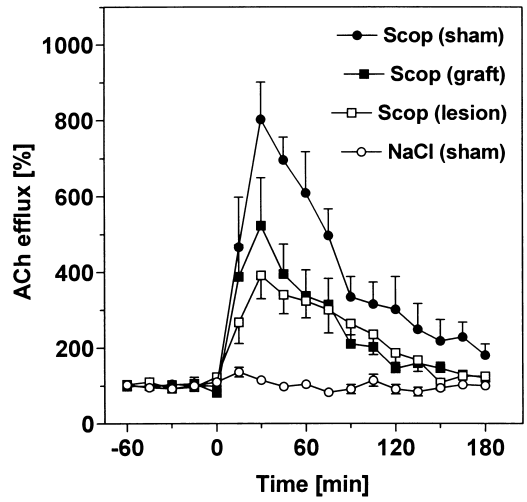


Fig. 2. Increase of hippocampal ACh release after administration of scopolamine (0.5 mg/kg i.p.) at time 0 to sham-operated (sham), lesion-only (lesion) and grafted (graft) rats. A control group of animals received saline injections (0.5 ml i.p.; NaCl). ACh efflux is expressed as percentage of the basal ACh release in each individual animal (the mean of values determined before drug administration was set at 100%). The data are given as means ± SEM of six experiments. Statistical analysis was done by two-way ANOVA for repeated measurements, sham vs. lesion ($F_{1,144} = 18.65$; $P < 0.001$); sham vs. graft ($F_{1,144} = 13.16$; $P < 0.001$); lesion vs. graft ($F_{1,144} = 0.33$; $P = 0.57$).

lesions [13]. This reasoning is supported by the fact that, in grafted rats, in which the AChE staining intensity was restored to control values, the basal release of ACh was significantly higher than in sham-operated rats (+57%; $P < 0.01$ vs. sham-operated rats; Fig. 1). As the ratio between the parameters 'AChE staining intensity' and 'basal ACh release' was similar in lesion-only and in grafted rats (Fig. 1), grafted neurons apparently exhibit a hyperactivity similar to the cholinergic fibres in the lesion-only animals. In other words, the altered regulation of ACh release in lesion-only animals was not restored after grafting.

The effects of lesioning and grafting on ACh release were further studied by pharmacological manipulations of transmitter release. Scopolamine (0.5 mg/kg i.p.), by inhibiting the autoreceptor on cholinergic terminals, induced an immediate increase of ACh release in all treatment groups which lasted for 2–3 h (Fig. 2). The clearcut increase of evoked ACh release in lesion-only animals demonstrates that the cholinergic fibres which remained after the FF lesion are responsive to presynaptic modulation. However, in lesion-only animals, the scopolamine-induced increases of ACh release were much reduced, compared to sham-operated animals. Thus, in lesion-only animals, the relative increase of ACh release was 4-fold, compared to 8-fold in sham-operated rats (Fig. 2). If expressed in absolute amounts, lesion-only animals released a maximum of 146 ± 23 fmol ACh/min while sham-operated animals released 399 ± 49 fmol ACh/min 30 min past scopolamine injection. In grafted animals, the relative increase of evoked ACh release was also smaller (5-fold; Fig. 2) than in sham-operated rats. This observation is in agreement with a recent study using brain slices in which a reduced sensitivity of presynaptic muscarinic autoreceptor was reported in grafted animals [2]. However, when ACh release is expressed in absolute terms, maximum ACh output in grafted animals (408 ± 98 fmol ACh/min) was not significantly different from sham-operated animals (399 ± 49 fmol ACh/min), and this finding underscores the functionality of muscarinic autoreceptors in grafted tissue.

In addition to the response of grafted tissue to autoreceptor blockade, we were also interested in the effects of serotonergic drugs on ACh release. The modulation of serotonergic systems has repeatedly been shown to affect cholinergic transmission and cognitive functions [1], and previous experiments had shown that co-transplantation of cholinergic and serotonergic tissue had more beneficial consequences for the recovery of cognitive functions than either graft alone [5]. For the present study, we chose to investigate the effects of 8-OH-DPAT, a serotonergic agonist acting specifically on 5-$HT_{1A}$ receptors. 8-OH-DPAT was previously shown to enhance ACh release in cortex and hippocampus of rats and guinea pigs (for review, see [1]). It must be noted that systemic 8-OH-DPAT is known to affect hippocampal ACh release via 5-$HT_{1A}$ receptors which are located postsynaptically in the target regions of the Raphe neurons. Thus, lesion studies indicated that hippocampal 5-$HT_{1A}$ receptors are located postsynaptically on

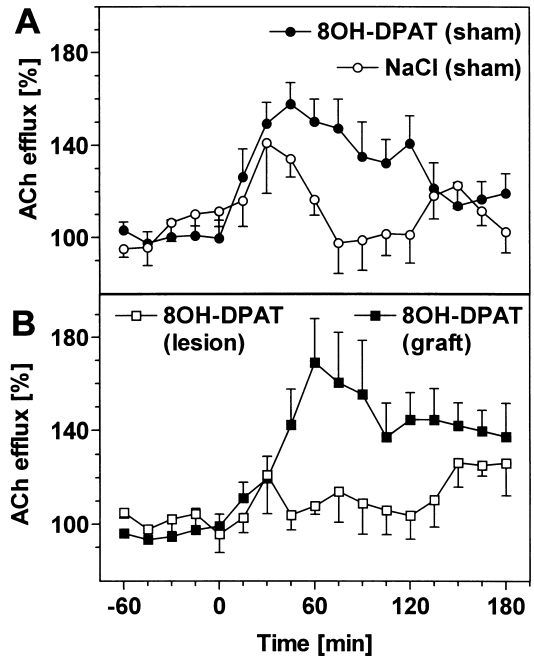


Fig. 3. Effects of 8-OH-DPAT administration (0.5 mg/kg s.c.) on hippocampal ACh release in sham-operated ((A), sham), lesion-only ((B), lesion) and grafted ((B), graft) rats. A control group of animals received saline injections (0.5 ml s.c.) ((A), NaCl). ACh efflux is expressed as in Fig. 2. The data are given as means ± SEM of six experiments. Statistical analysis was done by two-way ANOVA for repeated measurements, sham vs. NaCl ($F_{1,144} = 11.75$; $P < 0.001$); graft vs. lesion ($F_{1,144} = 17.24$; $P < 0.001$). Further statistical data are given in the text.

non-cholinergic and non-serotonergic neurons [1], and in microdialysis studies, the effect of 8-OH-DPAT on cortical ACh output was found to be mediated by dopamine [3].

In the present study, 8-OH-DPAT, given systemically, was found to significantly increase hippocampal ACh release (Fig. 3A; $P < 0.01$ vs. saline). The effect was maximal after 30–60 min and lasted for approximately 2 h. It should be noted that the injection of saline also leads to a short-lasting increase of ACh release which is due to an arousal reaction [11]. In lesion-only rats, 8-OH-DPAT did not affect ACh release and, remarkably, the ACh increase due to arousal was also absent (Fig. 3B). However, grafted animals fully reacted to 8-OH-DPAT (Fig. 3B), and the increase of ACh was not significantly different from the response seen in sham-operated animals (Fig. 3A) (statistical comparison between the responses in sham-operated and grafted rats: $F_{1,144} = 0.74$; $P = 0.39$ by two-way ANOVA for repeated measurements). Thus, the grafted rats have regained the ability to respond to 8-OH-DPAT administration with an increase of ACh release in the hippocampus. As 8-OH-DPAT affects ACh release by an indirect mechanism, the most likely explanation for this finding is that the grafted cholinergic neurons are sufficiently integrated into the neuronal circuitry of the host brain to respond to drugs which act in an indirect manner. In the present case, the grafted cholinergic neurons seem to be under control of afferents originating in the host brain and involving a serotonergic mechanism. This conclusion complements earlier studies by Nilsson et al. [11,12] who, using electrical and behavioral stimulations, observed that septal cholinergic neurons grafted into the denervated hippocampus are under control of dopaminergic and/or noradrenergic afferents [8].

In conclusion, the present study demonstrates that septal grafts lead to a restoration of ACh release in the hippocampus of fimbria-fornix lesioned animals. However, the high basal ACh efflux observed in grafted animals indicates a hyperactivity of these fibres, an observation which is also evident in the lesion-only animals and which may be due to a loss of endogenous inhibitory control. We report that grafted cholinergic neurons were sensitive to cholinergic autoregulation, and we demonstrate, to our knowledge for the first time, that the host brain exerts control on grafted cholinergic neurons by a serotonergic mechanism involving 5-$HT_{1A}$ receptors, a type of control which is also found in the septo-hippocampal system of intact rats.

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