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Lesion and stimulation of the ventral tegmental area increases cholinergic activity in the rat brain

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Our previous study indicated that unilateral lesion of the ventral tegmental area (VTA) facilitates contralateral VTA stimulation-induced feeding or exploration. The present study was aimed to determine the possible role of the central cholinergic systems in this effect. Immunohistochemistry for choline acetyltransferase (ChAT) was used to measure the number of active cholinergic neurons in their major groups (Ch1–Ch6) and in striatal regions in rats subjected to unilateral electrocoagulation and contralateral VTA electrical stimulation (L/S group) in comparison to the unilaterally stimulated (S), unilaterally lesioned (L) and sham (Sh) groups. The study showed that unilateral VTA lesion increased (as compared to Sh group) the number of $ChAT^+$ neurons in the Ch1–Ch3 and unilateral VTA stimulation increased the number in the Ch1 and the ventral pallidum only. The most sensitive to these changes in the mesolimbic system were cholinergic structures providing hippocampal afferentation. Surprisingly, there was no significant increase in the number of $ChAT^+$ neurons in the L/S group. The obtained results did not confirm any evident influence of the cholinergic systems on the VTA lesion-induced facilitation of the behavioral response evoked by contralateral VTA stimulation.

Key words: cholinergic system, ventral tegmental area, immunohistochemistry, choline acetyltransferase, stimulation, lesion

INTRODUCTION

The central cholinergic systems have widespread distant projections. On the basis of connectivity patterns, cholinergic systems are subdivided into six major groups of cells: Ch1–Ch6 (Mesulam et al. 1983). The basal forebrain (Ch1–Ch4) and the pontomesencephalic (Ch5–Ch6) groups are implicated in various cognitive functions, appetitive behaviors, waking-sleep regulation and generation of hippocampal theta rhythm (e.g. Vertes and Kocsis 1997, Sarter et al. 2003, Phillis 2005, Leszkowicz et al. 2007).

The present study is focused on functional relations between the cholinergic and mesolimbic systems. The mesolimbic dopaminergic system is involved in reward and in the regulation of various appetitive behaviors, including that produced by drugs of abuse (e.g. Le Moal and Simon 1991, Wise 2002). The ventral tegmental area (VTA), which is a key structure of the mesolimbic system, takes part in the controlling of food intake. Wyrwicka and Doty (1966) elicited immediate feeding in satiated cats by stimulation of the loci equivalent to the VTA and to the most posterior part of the lateral hypothalamus in rats, where according to Tohyama and Takatsuji (1998) dopaminergic A10 cells are localized. Our previous study indicated that unilateral lesions of the VTA facilitated behavioral responses, which was measured as shortening of the latency of feeding or exploration induced by electrical stimulation of the VTA in the contralateral hemisphere (Trojniar and Staszewska 1994, Trojniar and Klejbor 1999, Maliszewska-Ścisło and Trojniar 1999, 2000). The 'contralateral facilitation effect' appears immediately after lesioning (on the first post-lesion day), it is very stable and long-lasting (more than 2 months), and therefore it may constitute a not yet explored mechanism of lesion-induced brain plasticity.

The central cholinergic systems may potentially be involved in post-damage plasticity. Reorganization of cortical representations after cortical injury requires

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basal forebrain cholinergic input (Conner et al. 2005). The expansion of the rostral forelimb area evoked by rehabilitative training after cortical injury was eliminated in rats lacking the basal forebrain cholinergic system. Nishimura and coauthors (2002) found that electrolytic lesion of the basal nucleus of Meynert stunted plastic changes in the barrel cortex of neonatal mice after whisker removal. Another example of plasticity of the cholinergic system is the increase in the density of cholinergic fibers in the entorhinal cortex after immunotoxic lesion of the horizontal diagonal band of Broca (Hartonian and de Lacalle 2005).

The aim of this study was to examine whether the cholinergic system is involved in the plasticity connected with the facilitation of behavioral reaction, the facilitation being measured as shortening of the latency of feeding or exploration elicited by VTA stimulation after contralateral lesion of the VTA. The activity of the central cholinergic system was examined with the use of immunohistochemical detection of choline acetyltransferase (ChAT), which is an enzyme limiting the synthesis of acetylcholine. Neurons containing ChAT are considered to be cholinergic (e.g., Fibiger 1982). The number of ChAT positive neurons in brain structures can change after manipulation in the function of hormonal (e.g., Yamamoto et al. 2007), immunological (e.g., Beck et al. 2002) and nervous (e.g., Popović et al. 2006) systems. Hoover and coworkers (1978) found a high correlation (r=0.78) between ChAT activity and acetylcholine concentration in rat forebrain structures. Positive correlation between these two factors in most cholinergic brain areas was also found by Vizi and Palkowits (1978). The method of ChAT quantification seems to be valid in studying changes in the activity of the central cholinergic systems evoked by various experimental procedures. The number of active ($ChAT^+$) cholinergic cells was measured in the Ch1–Ch6 and striatal/pallidal structures after unilateral lesion and contralateral stimulation of the VTA ("contralateral facilitation effect" conditions) and in control groups subjected only to unilateral lesion or only to unilateral stimulation of the VTA as compared to the sham group.

METHODS

Animals and surgery

Male Wistar rats (n=20) weighing approximately 250–350 g at the time of surgery were used. They were housed in individual cages with free access to food and water under 12 h light/12 h dark illumination cycle with lights on at 06:00 AM. The animals were handled and adapted to the presence of an experimenter. All rats were implanted, under pentobarbital anesthesia (50 mg/kg, i.p.), with bilateral VTA electrodes (monopolar, stainless steel electrodes; 0.3 mm diameter, insulated on the entire length except for the flat-cut tip). Paxinos and Watson (1998) coordinates for the ventral tegmental area were used: 4.5 mm posterior to Bregma, 0.95 mm lateral to midline and 8 mm ventral to skull surface. The electrodes were anchored to four stainless steel skull screws with dental acrylic; stainless steel wire soldered to a screw served as the anode for electrical stimulation. The rats were assigned to four groups: L/S (n=5) subjected to unilateral lesion and contralateral stimulation of the VTA, L (n=5) subjected to unilateral VTA lesion, S (n=5) subjected to unilateral stimulation of the VTA, Sh (n=5) implanted with bilateral electrodes.

The experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and protocols were approved by Animal Research Ethical Committee.

Screening

After one-week recovery from the surgery, the L/S and S groups were screened for VTA stimulation-induced behaviors according to the method we described previously (Trojniar and Staszewska 1994, Maliszewska-Ścisło and Trojniar 2000). The aim of the screening was to determine individual (for each electrode) intensity of stimulating current which would, at a stimulation frequency of 50 Hz, induce behavioral response with a mean latency of 8–10 s. The testing was carried out for 4 days in a sound-attenuating chamber in a 250 × 350 × 440 mm box with food pellets scattered on the floor. Every day at 10:00 AM the rats were taken from their home cages and placed in the testing box where they had free access to food and were allowed to explore the box for 30 min before testing, to allow for habituation to the experimental conditions and for complete satiation. Trains of cathodal square-wave constant 0.1 ms current pulses were conducted from the stimulator (Hugo Sachs Elektronik, D7806 March F.R., Germany) to the electrodes by flexible wire leads. Pulse duration, pulse frequency and stimulation intensity were moni-

tored with an oscilloscope (Instek, GOS 620). Screening was carried out using a fixed stimulation frequency of 50 Hz. Current intensity was raised incrementally in 30-s trials (20 s rest between the trials) until subsequent exploration or eating were observed; the range of such intensity was 120–240 μA. Stimulation through each VTA electrode was tested in a separate block of trials; the one through which stimulation induced a more reliable behavioral response (feeding or exploration) was chosen as the stimulating electrode for further experimentation. Once determined, the particular stimulation intensity was used for all the subsequent tests.

Lesion

After completion of the screening procedure rats of the L/S and L groups were subjected to unilateral electrolytic lesion of the VTA under light ketamine anesthesia (85 mg/kg, i.p.). Cathodal current of 10 s duration and intensity of 1.0 mA was applied through the electrode contralateral to the stimulation electrode chosen during screening. Three of L/S rats received lesions in the right hemisphere and two in the left hemisphere.

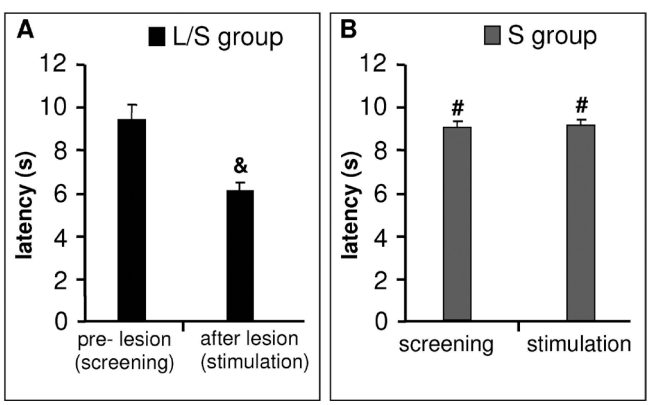

Fig. 1. Latency (mean ± SEM) of the VTA stimulation-induced behavioral response: (A) values before (screening: 1–4th day of stimulation with changeable current intensity) and after contralateral lesion of the VTA (stimulation: 5–14th day with fixed current intensity) (L/S group, n=5); (B) values during screening (1–4th day with changeable current intensity) and proper stimulation (5–14th day with fixed current intensity) in control, non-lesioned rats (S group, n=5). (&) P<0.01 significant difference between the latency of VTA stimulation-induced behavioral response after contralateral lesion and pre-lesion value (screening); (#) P<0.01 significant difference between the latency of VTA stimulation-induced behavioral response after contralateral lesion and non-lesioned S group during both screening and stimulation (Student's t-test).

Stimulation

The rats of the L/S and S groups were subjected to daily VTA stimulation for 10 days after the lesion (L/S group) or after completion of the screening procedure (S group). Stimulation conditions were identical to those during the screening except for the current intensity for the stimulation electrode, which was constant (fixed in screening). The latencies of the reactions were measured and averaged to obtain a mean latency for each rat. One hour after the stimulation, rats were overdosed with pentobarbital, brains were removed and subjected to histological procedures.

The rats of the L and Sh groups were sacrificed in the experimental room at the same time as the L/S and S groups (until then they stayed in their home cages).

Immunohistochemistry

Rats were transcardially perfused with saline followed by PBS containing 4% paraformaldehyde. The brains were removed and coronal sections of 30 μm thickness were made from each brain on a cryostat. The sections covered the rostro-caudal extension of the cholinergic systems [Bregma +1.6 to −9.14 according to the atlas by Paxinos and Watson (1998)]. The sections were washed three times with phosphate-buffered saline (PBS) and blocked for 45 min with a solution of 1% Bovine Serum Albumin, and then with 3% Normal Rabbit Serum in PBS with 0.1% Triton X at room temperature for effective reduction of nonspecific binding. The slices were then incubated with Polyclonal Goat Anti ChAT Antibody (Chemicon) at a dilution of 1:250 with PBS with 0.3% Triton X at 4ºC for 2 days. Afterwards the sections were washed three times with PBS with 0.3% Triton X and treated for 0.5 h with solution containing 3% Normal Rabbit Serum and 0.1% Bovine Serum Albumin in PBS. Then the sections were incubated for 1 h with Rabbit Anti Goat Biotinylated Antibody (Vector) at room temperature, washed three times with PBS, treated for 1 h with Vestastain solution (Vector) and stained using diaminobenzidine (DAB) enhanced with ammonium nickel sulfate. ChAT$^+$ cells somatas appeared dark brown or black.

Nissl staining

Nissl staining was carried out in those slices where according to the atlas of Tohyama and Takatsuji (1998) the dopaminergic A10 neurons are localized (ventral tegmental area and the posterior part of the lateral hypothalamus at sections 4.52 to 5.80 mm posterior to Bregma) to determine lesion and stimulating electrode placement. The sections were mounted on gelatin-coated slides, stained with Cresyl violet, dehydrated and finally mounted with DPX (Fluka).

Counting the labeled cells

The number of ChAT$^+$ cells was determined using the LeicaQWin software. The computer was coupled to a light microscope (Nikon Eclipse E600) equipped with a colour video camera (Leica DC 300). Cell counting was carried out at 40× final magnification in Ch1–Ch6 groups and in the striatal/pallidal structures in one section per animal on a fixed rostral-caudal level in relation to Bregma for each structure: medial septum and diagonal band of Broca (Ch1–Ch3): +0.2 mm, basal nucleus of Meynert (Ch4): −1.30 mm, pedunculopontine nucleus (Ch5): −8.00 mm, laterodorsal tegmental nucleus (Ch6): −8.72 mm, nucleus accumbens part shell and core (AcbS and AcbC): +1.2 mm, caudate-putamen part dorsal and ventral (CPuD and CPuV): −2.8 mm, globus pallidus (GP): −2.8 mm and the ventral pallidum (VP): +0.2 mm. ChAT immunoreactive neurons were quantified in the whole area of each structure in both hemispheres, cells were labeled on the monitor by clicking the mouse pointer on each cell and the Leica QWin software recorded the number of marks. The sections at the level of 4.52 to 5.80 mm posterior to Bregma (Nissl stained) were viewed at a 2.5 magnification to determine lesion and stimulating electrode placement. Borders of the structures were determined on the basis of Paxinos and Watson's atlas (1998).

Data analysis

The latency of behavioral response elicited by VTA stimulation was analyzed with a two-way analysis of variance (ANOVA) with the stage of stimulation (screening, stimulation) as a within-subjects factor and the group (lesioned L/S, non-lesioned S) as a between-subjects factor. The differences in the means were further analyzed with Student's *t*-test.

Statistical evaluation of the mean number of ChAT$^+$ cells was performed with a two-way ANOVA with treatment (lesion, stimulation, electrode implantation) as the between-subject factor and brain regions (12 levels) as a within-subjects factor. For comparison of Sh animals, whose brain hemispheres were both subjected to identical treatment, a one-way ANOVA with brain hemisphere (left, right) as a factor was performed. Since there was no significant interhemispheric difference ($F_{1,119}$=0.06, P>0.8), these data were combined to give one number for each tested brain region. The mean number of ChAT$^+$ cells in all four experimental groups (L/S, L, S, Sh) was compared separately in each tested brain region with an one-way ANOVA, and the differences in the means were further analyzed with Tukey's *post hoc* test; P<0.05 was considered significant.

RESULTS

Behavioral results

Electrical stimulation of the VTA performed in two stages [screening (1–4th day, current frequency: 50 Hz, rising current intensity), and proper stimulation (5th–14th day, current frequency: 50 Hz, current intensity fixed in screening)], evoked feeding (three of five L/S rats and two of five S rats) or exploration responses. Unilateral lesion of the VTA did not influence spontaneous behavior, with neither aphagia nor adipsia being found.

Two-way ANOVA (stage of stimulation × group) showed that the latency of the behavioral reaction changed across the two stages of stimulation $F_{1,19}$=12.59, P<0.01 and differed between lesioned (L/S) and non-lesioned (S) groups $F_{1,19}$=7.86, P<0.05. There was also significant ($F_{1,19}$=14.76, P<0.001) interaction between these factors. The mean latency of the behavioral reaction of L/S rats was 9.36 ± 0.6 s (mean ± SEM) in pre-lesion stimulation and was significantly (t_8=3.85, P<0.01) reduced to 5.89 ± 0.7 s after lesion of the contralateral VTA (Fig. 1A). Reduction of the latency was an index of the "contralateral facilitation effect".

The mean latency of the stimulation-induced behavioral response in the L/S rats after contralateral lesion of the VTA was shorter than that observed in the S rats, both

during the screening (9 ± 0.4 s, t_8=3.86, P<0.01) and the stimulation (9.13 ± 0.3 s, t_8=3.86, P<0.01, Fig. 1A, B).

Number of ChAT positive neurons

Two-way ANOVA (treatment × brain region) showed that the experimental treatment (lesion, stimulation or electrode implantation) significantly influenced the number of ChAT$^+$ cells ($F_{2,419}$=3.84, P<0.05) which differed in the various brain regions ($F_{11,419}$=57.32, P<0.001). The interaction between these factors was not significant ($F_{22,419}$=0.66, P>0.87).

ChAT positive brain structures were grouped into two functional units: major groups of cholinergic cells (Ch1–Ch6) (Fig. 2) and striatal/pallidal cholinergic interneurons (Fig. 3) in nucleus accumbens, part shell (AcbS), nucleus accumbens, part core (AcbC), dorsal part of the caudate-putamen (CPu-D), ventral part of the caudate-putamen (CPu-V), globus pallidus (GP) and the ventral pallidum (VP).

One-way ANOVA showed that the number of ChAT$^+$ cells differed significantly between groups in the following brain areas: Ch1 ($F_{6,34}$=3.552, P<0.01), Ch2 ($F_{6,34}$=3.23, P<0.05), Ch3 ($F_{6,34}$=2.85, P<0.05), CPuV ($F_{6,34}$=3.30, P<0.05) and VP ($F_{6,34}$=2.53, P<0.05).

Tukey's test (P<0.05) showed that unilateral lesion of the VTA (L group) bilaterally increased the number of ChAT$^+$ cells in the Ch1–Ch3 major groups of cholinergic cells in comparison with the Sh group. Unilateral stimulation was effective in increasing the number of these cells in the Ch1 bilaterally (Fig. 2) and in VP in the stimulated hemisphere (Fig. 3). There was no significant difference among L/S, L and S groups.

A tendency (not statistically significant) of an increase in the number of ChAT$^+$ neurons was observed after all procedures: lesion and contralateral VTA stimulation (L/S group), unilateral lesion of the VTA (L group) and unilateral VTA stimulation (S group) especially in the Ch1–Ch3 (Figs 2 and 4) and Ch6 (Fig. 2) and in the VP (Fig. 3).

Localization of lesions and the tips of stimulating electrodes

Histological verification (Fig. 5) showed that VTA was damaged in its anterior (Bregma: −4.80 mm) and central (Bregma: −5.20 to −5.30 mm) part. In two cases, lesions encroached the transition area between the most posterior portion of the lateral hypothalamus (LH) and the VTA (Bregma: −4.52 mm), where according to

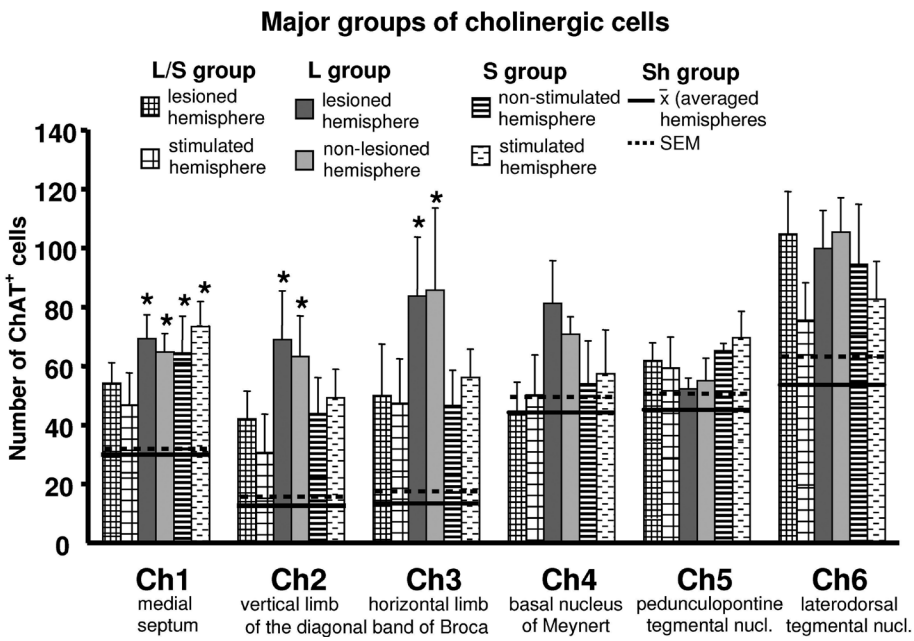

Fig. 2. Number of ChAT$^+$ cells (mean ± SEM) in the major groups of cholinergic cells (Ch1–Ch6) after lesion and contralateral stimulation of the VTA (L/S), unilateral lesion of the VTA (L) and unilateral stimulation of the VTA (S) in comparison with the sham (Sh) group. Continuous horizontal line on bars – the mean number of ChAT$^+$ cells in the Sh rats averaged in left and right brain hemisphere; dashed line – SEM; (*) significant (P<0.05) difference in comparison with the Sh group (Tukey's test).

Tohyama and Takatsuji (1998) dopaminergic A10 neurons are also situated. The tips of stimulating electrodes were found in the whole rostro-caudal extension of the VTA (Bregma: −4.80 to −5.60 mm) and also in the area between the VTA and LH (Bregma: −4.52 mm).

DISCUSSION

The obtained results can be summarized as follows: (1) unilateral lesion (L group) increased the number of ChAT$^+$ neurons in the medial septum and diagonal band of Broca (Ch1–Ch3) (Fig. 2); (2) unilateral stimulation (S group) increased the ChAT- immunoreactivity only in the medial septum (Ch1) (Fig. 2) and the ventral pallidum (VP) (Fig. 3); (3) no significant increase in the number of ChAT$^+$ cells during the "contralateral facilitation effect" was observed in the L/S group (Figs 2–3). (4) There were no differences among experimental (L/S, L and S) groups and no interhemispheric difference within any group (Figs 2–3).

The study shows global changes in the cholinergic system after electrolytic lesion or electrical stimulation of a non-cholinergic structure, which is the VTA. While there was a reduction of latency of stimulation-induced behavioral response after contralateral VTA lesion (L/S group), the number of ChAT$^+$ cells during the "contralateral facilitation effect" did not differ significantly from that obtained after single procedures essential for its triggering (lesion or stimulation alone). It seems that the cholinergic system is not involved in the mechanism responsible for this phenomenon.

There is much evidence that specific lesion of the cholinergic structures causes loss of cholinergic fibers and acetylcholinesterase (AchE) or ChAT activity in the efferent structures (e.g., Naumann et al. 1997, de Lacalle et al. 1998, Gu et al. 1998, Waite and Chen 2001, Birthelmer et al. 2003, Popović et al. 2006). However, there are only a few studies on changes in the cholinergic deafferented structures after lesion of non-cholinergic structures (Henderson et al. 1998, Muma et al. 2001, Zhang et al. 2008) and no study on the number of ChAT$^+$ neurons after VTA lesioning. According to current data, there is no study addressing the number or density of ChAT$^+$ cells after electrical brain stimulation so the present study is the pioneer one.

In this study, unilateral lesion of the VTA had the strongest influence on the number of ChAT$^+$ neurons. (Figs 2–3). We used electrocoagulation of the VTA, which is a non-specific lesion, damaging neurons of various neurotransmitter systems. However, the VTA provides mainly dopaminergic projections to the cholinergic neurons of the basal ganglia (Gaykema and Zaborszky 1996). We observed that most of the significant differences between the experimental and sham groups appeared mainly in the medial septum and diagonal band of Broca (Ch1–Ch3) (Fig. 2). These structures innervate the hippocampal formation (e.g., Vertes and Kocsis 1997). Robinson and coauthors (1979) found increased acetylcholine turnover rate in the hippocampus after 6-hydroxydopamine chemical lesion of A10 dopaminergic cells or their terminals in the septum. From the above study it emerges that dopaminergic neurons exert a tonic inhibitory effect on the cholinergic metabolism of the septohippocampal pathway. The inhibitory effect is transynaptically

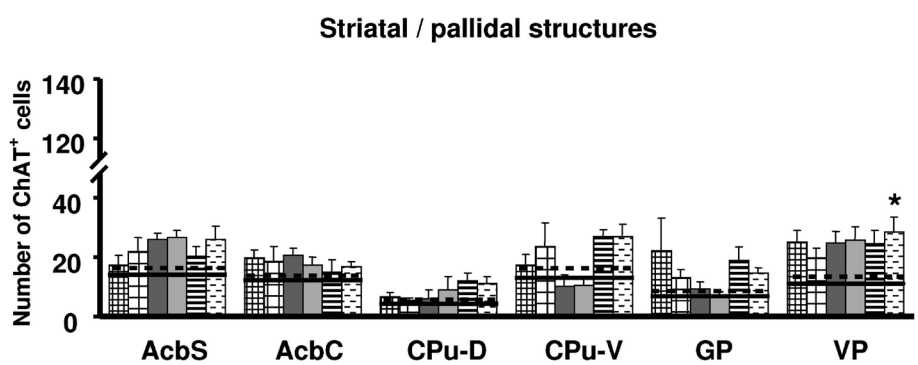

Fig. 3. Number of ChAT$^+$ interneurons (mean ± SEM) in the striatal/pallidal structures after lesion and contralateral stimulation of the VTA (L/S), unilateral lesion of the VTA (L) and unilateral stimulation of the VTA (S) in comparison with the sham (Sh) group. (AcbS) nucleus accumbens, part shell; (AcbC) nucleus accumbens, part core; (Cpu-D) dorsal part of the caudate-putamen; (Cpu-V) ventral part of the caudate-putamen; (GP) globus pallidus; (VP) ventral pallidum. For other explanations see Fig. 2 legend.

mediated *via* γ-aminobutyric acid (GABA)-ergic interneurons (McLennan and Miller 1974, Assaf and Miller 1977). Dopamine released from A10 cells stimulates the GABA-ergic interneurons, which in turn inhibit the cholinergic septohippocampal neurons (Le Moal and Simon 1991). Tonically inhibited "silent" cholinergic neurons of Ch1–Ch3 groups (as those in our control Sh group) apparently do not synthetize ChAT. Lesion of the VTA performed in our L and L/S groups probably disinhibited cholinergic septohippocampal neurons which consequently became active and started producing ChAT.

The suppressive effect of dopaminergic transmission on cholinergic neurons is not limited to the septohippocampal pathway. Muma and coauthors (2001) found that removal of dopaminergic inputs to the forebrain results in hyperactivity of the Ch4 group of cholinergic cells in the basal nucleus of Meynert. The study of Zhang and colleagues (2008) indicated that cholinergic and non-cholinergic neurons in the pedun-

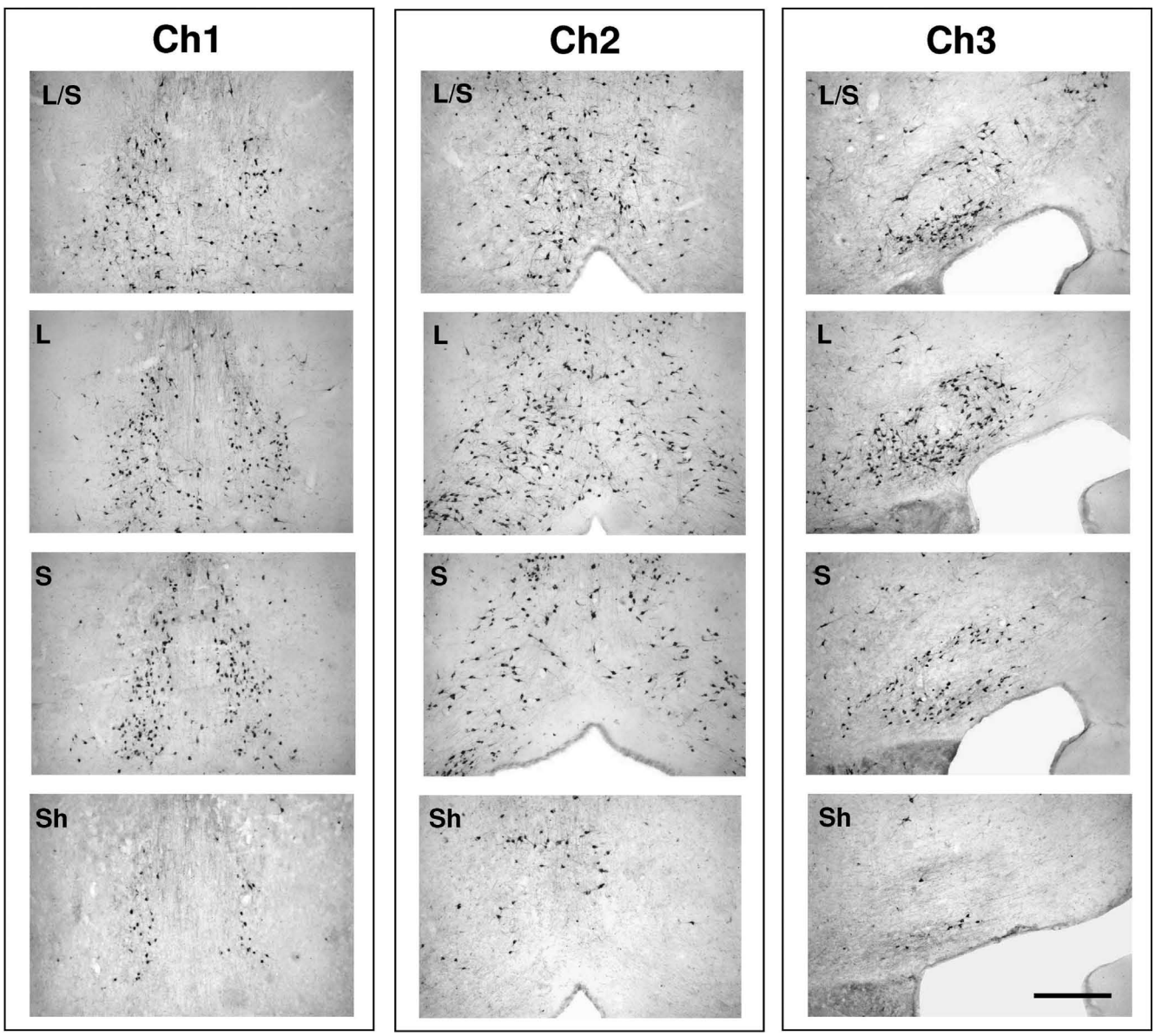

Fig. 4. ChAT$^+$ neurons in the (Ch1–Ch3) major groups of cholinergic cells after lesion and contralateral stimulation of the VTA (L/S), after unilateral lesion of the VTA (L), after unilateral stimulation of the VTA (S) and after bilateral electrode implantation in a representative sham (Sh) rat. The symbol of the experimental group is in the left top corner of each photograph. Cholinergic groups of cells are located in: Ch1 – medial septum; Ch2 – vertical limb of diagonal band of Broca; Ch3 – horizontal limb of diagonal band of Broca. Scale bar is 500 μm.

culopontine nucleus are overactive in 6-hydroxydopamine-lesioned rats. In the present study, only an insignificant tendency of the number of ChAT$^+$ cells to increase in Ch4 group, and no changes in the Ch5 cells, were observed after unilateral electrocoagulation of the VTA. Our data seem to be consistent with the previous data of Muma and others (2001) but not Zhang and coworkers (2008). The reason for this discrepancy could be the different kind of lesion: specifically, chemical lesion of dopaminergic and noradrenergic neurons in the study of Muma and colleagues (2001) and Zhang and others (2008) and nonspecific electrocoagulation in our study.

Interestingly, unilateral stimulation of the VTA alone (in the S group) induced the same effect as unilateral lesion of the VTA (in the L group), i.e., increased a number of ChAT$^+$ cells in the region of the medial septum (Ch1) (Fig. 2). The equivalence between the effects of lesion and stimulation could be easily explained if the electrical stimulation itself produced electrolysis. However, an occurrence of such an effect in the present study is doubtful. A single stimulatory impuls lasts for merely 0.1 ms and is followed by a 19.9 ms break. The current parameters and electrode polarization were controlled with an oscilloscope. The short time of a single impulse and relatively low amplitude of cathodal current prevented electrolysis as could be seen in Fig. 5F. We did not observe any tissue damage under the tip of the stimulating electrode while there is a distinct loss of tissue bordered by electrocoagulated tissue in the trace of lesioning (1.0 mA, 10 s cathodal current) electrode. There are many studies where similar parameters of stimulation were applied and stimulation did not evoke electrolysis (Dean and Kostreva 1987, Trojniar and Staszewska 1994, Wyrwicka and Doty 1996, Hashimoto et al. 1998, Maliszewska-Ścisło and Trojniar 1999, Nishihama et al. 1999, Trojniar and Klejbor 1999, 2000, Simon et al. 2008). The equivalence between the effects of lesion and stimulation could result from the structure of the neuronal circuit. The dopaminergic system influences the septal cholinergic neurons *via* the GABA-ergic neurons, which send their inhibitory collateral fibers either back to themselves or to other GABA-ergic cells within the septum, and occasionally form axo-axonal synapses on terminals of septal afferents (e.g. dopaminergic fibers from VTA). The negative feedback can lead to disinhibition of septal cholinergic neurons both after removal (VTA lesion) and increasing (VTA stimulation) of dopaminergic transmission to septum. There is only limited experimental evidence that lesion and stimulation evokes an identical effect. Our previous findings (Maliszewska-Ścisło and Trojniar 1999) showed that both unilateral lesion and unilateral stimulation of the VTA facilitated feeding response induced by contralateral VTA stimulation. Another example of similar effects of lesion and stimulation concerns the treatment of Parkinson's disease. It has been demonstrated that lesions of the subthalamic nucleus (STN) alleviate motor deficits in parkinsonian monkeys (e.g., Bergman et al. 1990, Aziz et al. 1991). Other data showed that high frequency stimulation of the STN evoked the same effect: improved motor function (e.g., Benazzouz et al. 1996, Darbaky et al. 2003). Moreover, the same immunosuppressive effect following both electrical stimulation and lesion of the ventromedial hypothalamus was observed in rats (Okamoto et al. 1996, Wrona and Trojniar 2005). In addition, our previous data (Prabucka et al. 2005, 2006) indicated that both unilateral lesion and contralateral stimulation of the VTA evoked increasing effect on neuronal activity measured with *c-fos* expression in many prosencephalic and diencephalic brain structures. There was no difference in *c-fos* expression between lesioned and stimulated hemispheres.

In the present study most of the significant differences between the experimental and sham groups were found in the hippocampal afferenting structures (Ch1–Ch3 in the medial septum and the diagonal band of Broca) (Fig. 2). Thiel and coauthors (1998) found that exposure of rats to a novel environment led to increased extracellular levels of hippocampal acetylcholine. Re-exposing animals to the same environment did not indicate habituation. The possibility that the novelty stress could be the cause of the increase in the number of septohippocampal ChAT$^+$ neurons in the present study must be discussed. In this research, rats of L/S and S groups were taken (for 10 consecutive days) from their home cages to the laboratory room and placed in the experimental cages, where stimulation was performed. Rats of the L group were placed in the experimental cages once, when the lesion was performed, and finally rats of the Sh group stayed in their home cage during the entire experiment. The significant increase in the number of ChAT$^+$ cells in Ch1–Ch3 in rats of the L group (Fig. 2) could be a result of acute stress evoked by both lesion and the single removal from the home cage friendly environment. However, measurement of cholinergic activity was

performed 10 days after VTA lesioning. Therefore, it seems that it is the post-damage plasticity that influences cholinergic activity and not the effects of acute stress. Similar results were obtained by Henderson and colleagues (1998), who found that mechanical lesion (microknife cut) of the entorhinal cortex evoked an increase in the density of $ChAT^+$ terminal boutons in the dentate gyrus of the hippocampus 10 days after lesioning, which could be a result of sprouting of the cholinergic axons. Some authors (Waite and Chen 2001, Chang and Gold 2004) showed that lesions of the cholinergic cells in the basal ganglia increase the rate of acetylcholine synthesis and proliferation of $ChAT^+$ fibers in denervated hippocampus, which could be additional evidence for the participation of the cholinergic systems in the post-damage plasticity.

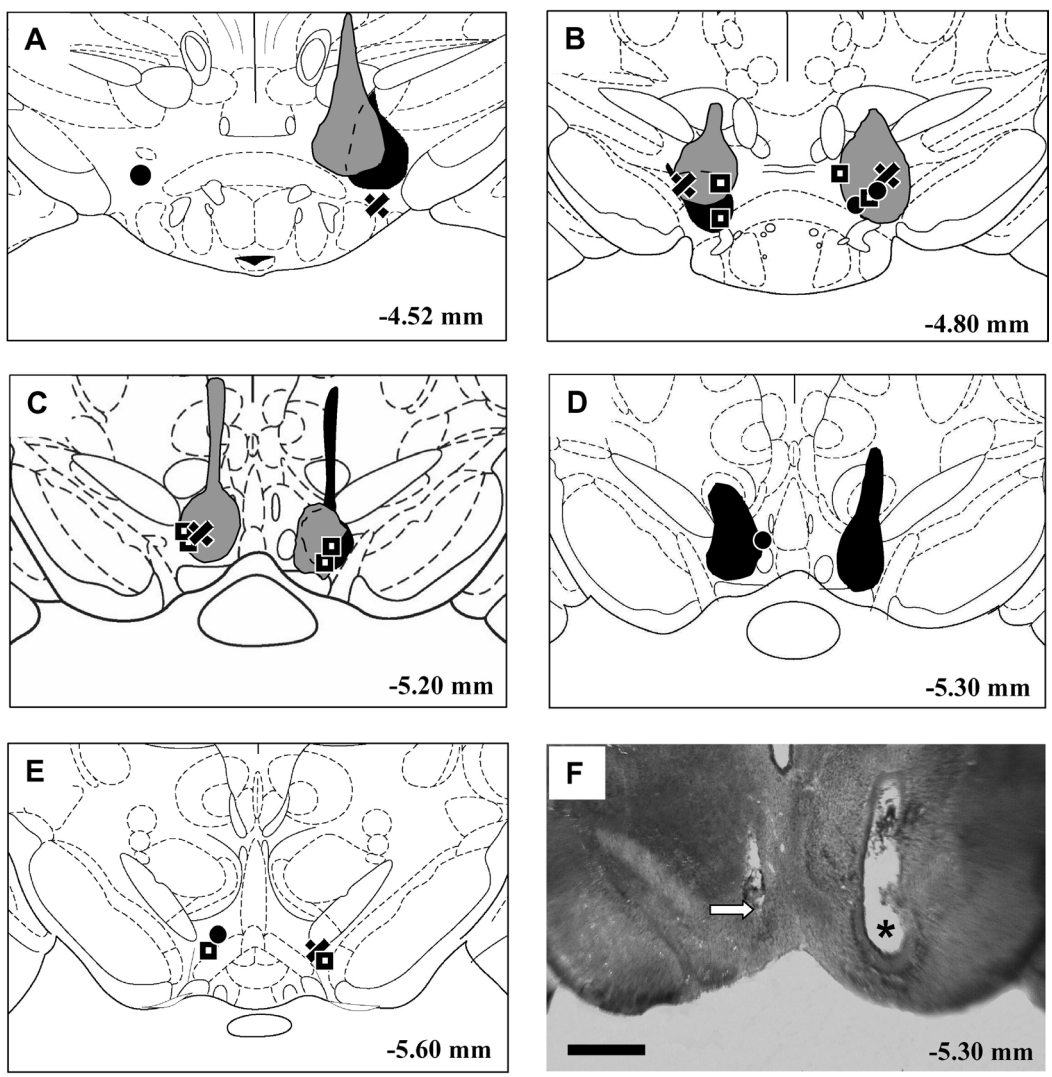

Fig. 5. Localization of lesions and the tips of stimulating electrodes; (A–E) maximal extension of the lesions in the L/S (black areas) and in the L (gray areas) rats, localization of stimulating electrodes in the L/S (black dots) and in the S (black crosses) rats and localization of the sham electrodes in rats of the Sh group (black squares). Plates were taken from atlas by Paxinos and Watson (1998)* and framed to show the ventral tegmentum. The distance from Bregma of each plate is shown in the bottom right corner; (F) a photograph of the Nissl stained brain section (framed to the ventral tegmentum) of a representative L/S rat. The tip of stimulating electrode is marked with a white arrow. The trace of the lesioning electrode bordered by electrocoagulated tissue is marked with a black asterisk. Scale bar is 1 mm.

* Reprinted from Paxinos G, Watson C, The Rat Brain in Stereotaxic Coordinates, 4th Ed., Figures 38–42, Copyright (c) 1998, Academic Press Inc. San Diego CA, with permission from Elsevier

In the present study, activity of the cholinergic striatal interneurons was also examined in the conditions of lesion and stimulation of the VTA. After stimulation of the VTA (S group), the number of $ChAT^+$ cells increased in the ventral pallidum (VP) only ipsilaterally (Fig. 3). In the electrophysiological study of Nanda et al. (2009), stimulation of the centromedian (CM) thalamic nucleus decreased firing rate in most of the cholinergic striatal interneurons and reduced acetylcholine levels in striatum. CM provides excitatory glutamatergic projections to the striatum (e.g. Smith et al. 2009), while neurons of the VTA, which was stimulated in the present study, release inhibitory mediators (dopamine or GABA) (Thierry et al. 1980, Le Moal and Simon 1991, Tzschentke and Schmidt 2000) so the present results seem to be consistent with the results obtained by Nanda and others (2009).

CONCLUSIONS

The obtained results do not support our hypothesis that the cholinergic system is directly involved in the VTA post-damage facilitation of behavioral responses elicited by stimulation of the contralateral VTA. Although our data show that both procedures (lesion or stimulation of the VTA) may increase the number of active cholinergic cells, the effect of lesion is stronger. The structures most sensitive to the changes of the mesolimbic activity are the ones providing cholinergic projections to the hippocampus.

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