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Research report

Ventral tegmental area dopaminergic lesion-induced depressive phenotype in the rat is reversed by deep brain stimulation of the medial forebrain bundle

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HIGHLIGHTS

- Results confirm the feasibility of chronic, continuous, bilateral MFB-DBS.
- Bilateral dopamine lesion of the VTA produce depressive-like phenotype in rats.
- MFB-DBS can reverse depressive-like phenotype.
- MFB-DBS activates distant structures involved in the neurocircuitry of depression.
- MFB-DBS can act via both DA dependent or independent mechanisms.

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ABSTRACT

DBS of the medial forebrain bundle (MFB) has been investigated clinically in major depressive disorder patients with rapid and long-term reduction of symptoms. In the context of chronic bilateral high frequency deep brain stimulation (DBS) of the MFB, the current study looked at the impact of lesioning the ascending dopaminergic pathway at the level of the ventral tegmental area (VTA). Sprague-Dawley female rats were given bilateral injection of 6-OHDA into the VTA (VTA-lx group) or were left unlesioned (control group). Later, all animals received bilateral microelectrode implantation into the MFB followed by chronic continuous stimulation for 3 weeks. Behavioral tests were performed as baseline and following MFB-DBS, along with histological analysis. Pre-stimulation baseline testing of the VTA-lx animals indicated depressive-like phenotype in comparison with controls. Response to MFB-DBS varied according to (i) the degree of dopaminergic depletion: animals with severe mesocorticolimbic dopamine depletion did not, whilst those with mild dopamine loss responded well to stimulation; (ii) environmental conditions and the nature of the behavioral tests, e.g., stressful vs non-stressful situations. Neuromodulation-induced *c-fos* expression in the prelimbic frontal cortex and nucleus accumbens was also dependent upon integrity of the dopaminergic ascending projections.

Our results confirm a potential role for dopamine in symptom relief observed in clinical MFB-DBS. Although mechanisms are not fully understood, the data suggests that the rescue of depressive phenotype in rodents can work via both dopamine-dependent and independent mechanisms. Further investigations concerning the network of depression using neuromodulation platforms in animal models might give insight into genesis and treatment of major depression disorder.

1. Introduction

During the last decade electric stimulation of core brain structures, in the form of deep brain stimulation (DBS), has been considered in the context of psychiatric diseases [1]. Several clinical trials targeting various regions associated with depression pathology have been conducted in major depressive disorder (MDD) [2–5].

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In a recent clinical trial the supero-lateral branch of the medial forebrain bundle (slMFB) was stimulated which projects and directly interacts with all the previously selected targets such as the nucleus accumbens, subgenual cingulate cortex, and the ventral capsule/ventral striatum [2,6–9]. Pre-clinical data has well established that the MFB, mediated by dopamine, sustains reward orientated behavior, such as motivation, drive and "wanting" [10–12]. Clinically, slMFB stimulation, likely by modulating dopamine transmission that impacts on all the other previously selected downstream targets, produced rapid and chronic antidepressive effects at low stimulation intensity, yielding a response rate of 85%. Among them, four out of six patients qualified as remitters [4,13]. Taken together, these preliminary findings suggest that the MFB might be a promising target for neuromodulation in refractory depression, and has spurred on the need for further pre-clinical investigation in experimental models, of both (i) the pathophysiology of depression and (ii) the mechanisms underlying MFB neuromodulation in rescuing anhedonic and depressive symptoms [3].

The current study combined stereotactic lesioning and chronic and continuous DBS in rodents [14], in order to investigate the impact of bilateral dopamine depletion from the mesocorticolimbic circuitry and subsequent MFB-DBS. Our findings confirm the role of ascending VTA dopaminergic projections in the maintenance and modulation of affective behavior. The lesion of VTA dopamine neurons, leading to dopamine depletion in the nucleus accumbens, produced depressive-like phenotype in the animals, which was – in some circumstances – reversed by MFB-DBS. Furthermore, stimulation mediated reversal of phenotype was shown to differentially affect animals depending on the severity of the depletion: animals with severe mesocorticolimbic dopamine depletion did not, whilst those with mild dopamine loss responded well to stimulation. The data strengthens the concept of the MFB as a robust target for neuromodulation in the treatment of depression, and implicates dopamine as substrate mediating the effect.

2. Methods and materials

2.1. Study design

The design is summarized in Fig. 1. Young adult female Sprague-Dawley (SD) rats ($n = 21$, Charles River, Germany), weighing 250 g, were housed in individual round cages (height: 40 cm; diameter: 40 cm), with the light/dark cycle maintained at 12 h on and 12 h off with food and water available ad libitum . After two weeks of handling and habituation, rats ($n = 11$) received bilateral stereotactic injection of 6-OHDA in the ventral tegmental area (VTA), aiming to deplete dopamine in the mesolimbic and mesocortical pathways. A control group ($n = 10$) remained unlesioned. Following 1 month, all animals were submitted to baseline behavioral test sessions and then bilaterally implanted with stimulating microelectrodes in the MFB. Phenotypic assessment was done using validated test sensitive to depressive-like behavior such as the sucrose preference test (SPT), the forced-swim test (FST), and Ultrasonic Vocalization (USV). Following a recovery period of 2 weeks, chronic continuous bilateral MFB-DBS was applied for 3 weeks to both groups. Finally, a post-stimulation round of SPT, FST and USVs was performed and animals were transcardially perfused for histological analysis. The study described in this manuscript had the approval of the veterinary board for research in animals of the University of Freiburg (TVA G10-124) and was carried out in accordance with the EU Directive 2010/63/EU concerning the protection of animals used for scientific purposes.

2.2. Surgical procedures and deep brain stimulation

Rats underwent general anesthesia induced and maintained by inhalation of Isoflurane 2%. First, 6-OHDA (3.6 μg/μl in 0.2% ascorbic acid and 0.9% saline; Sigma, USA) was bilaterally injected in the VTA at the following coordinates relative to bregma: Anterior–posterior (AP) = −5.1; medio-lateral (ML) = −0.6; dorso-ventral (DV) = −7.4 (total volume of 2.0 μl). In a second time-point, bipolar electrodes (125 μm diameter each, 90% platinum/10% iridium Teflon-coated, World Precision Instruments, Sarasota, USA) were stereotaxically bilaterally implanted into the MFB: AP = −4.4, ML = ±1.2, DV = −7.8 and permanently fixed to the skull surface with microscrews and bone cement as previously described elsewhere [14]. A single shot of buprenorphine (75 μg/Kg, i.p.) was given to all animals for post-operative analgesia. Two weeks of recovery followed surgery before animals were connected to a pulse generator (STG 2008, Multichannel Systems, Germany) for stimulation (square-wave biphasic constant current, 130 Hz, 100 μs and 250 μA average current). Current was individually titrated for each cerebral hemisphere, beginning with 50 μA to a maximum of 350 μA, or to a level just before inducing side effects, i.e., rotational movement, which indicate the electric field to encroach onto the cerebral peduncle in the vicinity of the MFB. Chronic and continuous MFB-DBS was performed for 3 consecutive weeks [15,16].

2.3. Behavioral and histological assessment

Behavioral test sessions were performed at baseline (4 weeks following bilateral 6-OHDA injection) and at post MFB-DBS (by the end of the third week of continuous MFB stimulation).

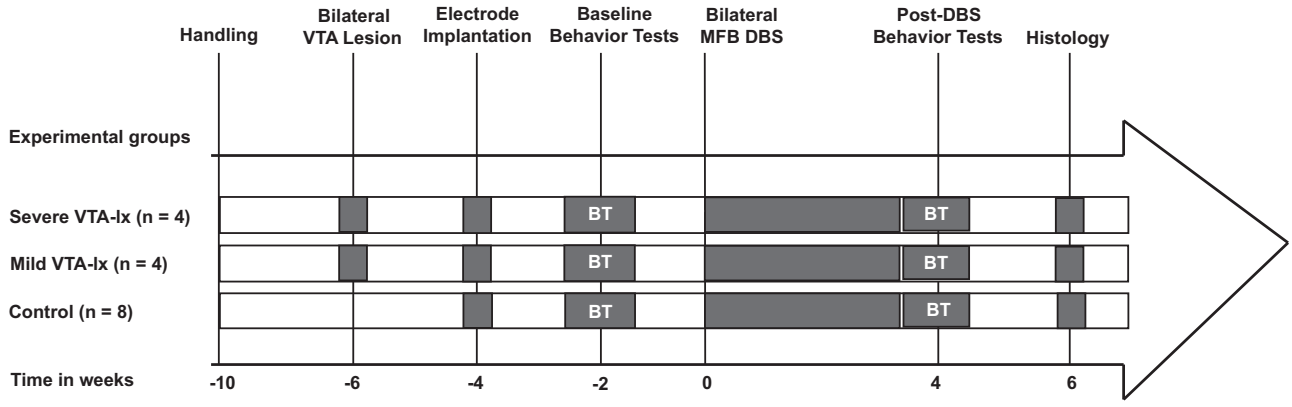

Fig. 1. Study groups and milestones of the experiment.

2.3.1. Ultrasonic vocalization test

Rats were placed in individual Plexiglas cages (identical to the home-cages) adapted with the recording microphones, sensitive to frequencies of 15–100 kHz, 60 cm above the bottom, connected to an Ultrasound Gate interface (Metris, Netherlands) and a computer. Vocalization was monitored in two frequency bands: a low band of 19–25 kHz, associated with negative affect; and a High band of 47–53 kHz, associated with positive affect. Stable environmental conditions were maintained during the 2 h of continuous recording. Behavior analysis was performed using an automated evaluation system (Sonotrack 2.0, Metris, Netherlands). The number of events that occurred in each frequency range during the experiment was acquired for statistical assessment [16,17].

2.3.2. Forced-swim test

FST is a well-established behavioral assessment of distress in animal models of depression, and used to evaluate antidepressant effect of drugs or other treatments in rodents [3,15,18,19]. In this study, a single exposure FST paradigm was applied, aiming to avoid biased memory processed increase of immobility, as discussed elsewhere [16,20,21]. The rat is placed into a cylindrical receptacle (40 cm high, 20 cm diameter) filled with water (25 °C), so that it could neither touch the bottom of the container nor escape. Behavioral activity was recorded for 7 min by a digital video camera connected to the workstation (Viewer2, Biobserve, Germany). The amount of time spent in a posture of immobility was calculated.

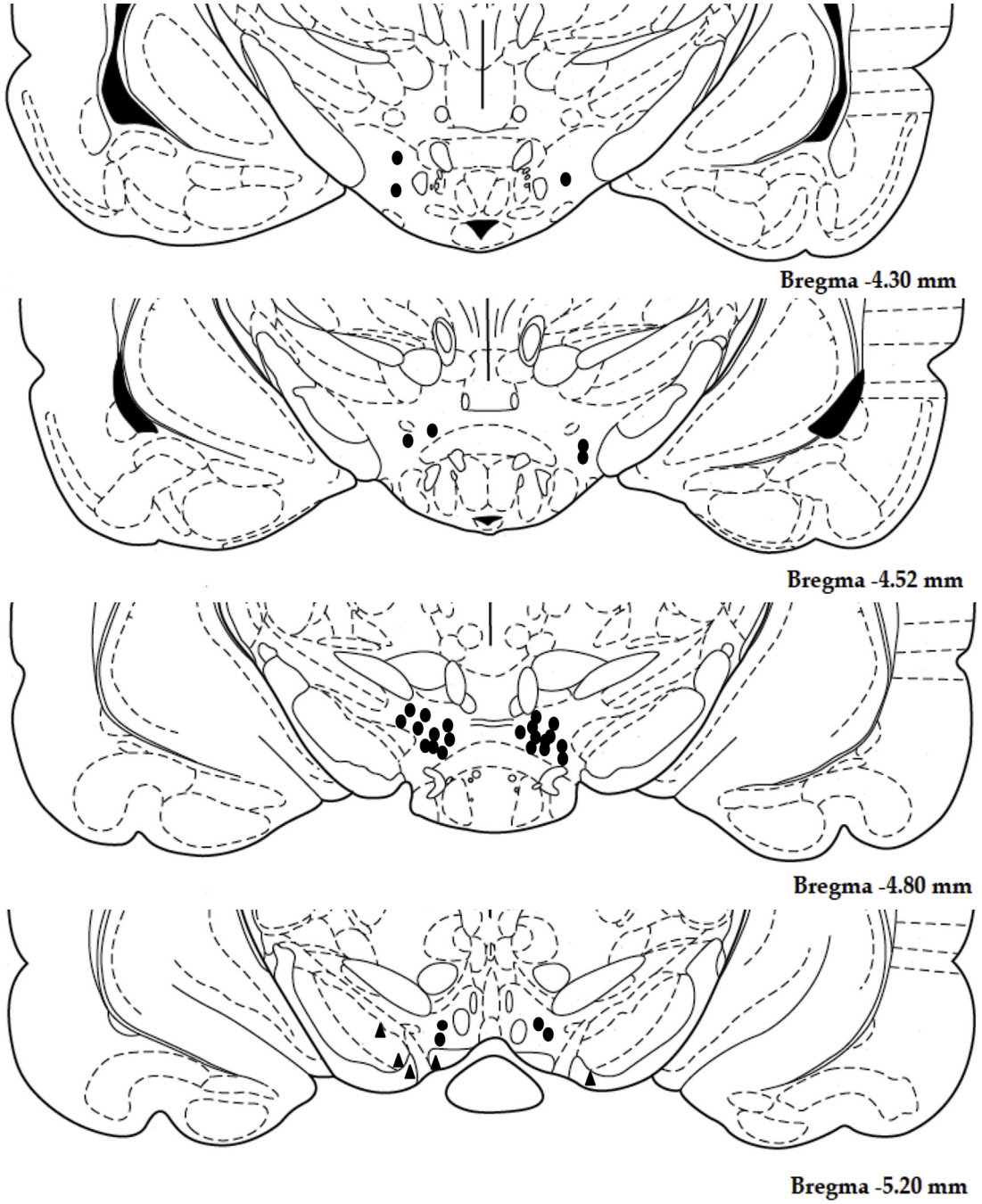

Fig. 2. Schematic representation of bilateral electrode placement in the medial forebrain bundle in coronal sections of the rat brain. Black dots indicate the tip of electrodes into the MFB. Triangles represent misplaced electrodes. Permission to reprint figures from The Rat Brain Atlas in stereotaxic coordinates, 3rd Edition, Paxinos and Watson, 1997, granted by Elsevier.

Immobility was defined as: (i) no movement of the three out four paws, (ii) no struggling, (iii) floating behavior.

2.3.3. Sucrose preference test

Anhedonic reactivity is typically evaluated in the rat as a decrease in sweet solution intake or in its preference in a two-bottle test [22,23]. During this test, rats were offered for 24 h 2 bottles containing 400 ml, one with 5% sucrose solution and the other tap water. Prior to the test, animals were not deprived of food or water. The amount of consumed water and sucrose solution was then calculated and presented as a percentage of the total volume in relation to the body weight at the time of the behavioral assessment.

2.3.4. Elevated plus maze

Anxiety behavior was assessed by the elevated plus-maze test, according to a standard protocol [24]. The animals were placed in the center of the maze, consisting of two open arms and two enclosed arms. The amount of time spent in the closed arms (in percent) and the number of entries into the closed arms over 5 min were assessed (Viewer2, Biobserve, Germany).

2.4. Immunohistochemistry and histological analysis

Following the last round of USVs animals were terminally anesthetized by an overdose of 10% ketamine (Bela-Pharm GmbH & Co., KG, Germany) and 2% xylazine (Rompun, Bayer-Leverkusen, Germany) and intracardially perfused with a solution containing 4% paraformaldehyde and 0.025% glutaraldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were removed from the skull, kept in 30% sucrose at 4 °C until they sunk, and cut into 40 μm coronal sections. The free-floating sections were incubated with 1.5% H_2O_2 and 1% sodium-borohydride, each in 0.02 M sodium phosphate buffer at pH 7.4 for 30 min, and exposed to a primary antibody raised in goat against *c-fos* (SC-52-G, 1:2000, Santa Cruz Biotechnology Inc., Santa Cruz, USA), or mouse anti-tyrosine hydroxylase (TH) (1:2500; Sigma–Aldrich, Steinheim, Germany), or rabbit anti-serotonin (1:2000; Sigma S5545, Darmstadt, Germany). After incubation for 48 h at 4 °C, visualization of antibody-binding sites was based on DAB staining using biotinylated anti-goat (BA-5000; 1:200; Vector Laboratories, Inc., Burlingame, USA) or biotinylated anti-mouse (BA-2001; 1:200; Vector Laboratories, Inc., Burlingame, USA) as secondary antibody and avidin-biotin-technique (ABC Elite; Vector Laboratories, Burlingame, CA) for signal intensification. Finally 3,3′-diaminobenzidine (DAB; Merk, Darmstadt, Germany) and 0.01% H_2O_2 were used to develop the color reaction. The sections were mounted on super frost plus slides (Langenbrinck, Emmendingen, Germany), dehydrated in ascending alcohol solutions, and cleared in xylene before they were cover slipped with Histofluid (Marienfeld Laborglas, Lauda-Königshofen, Germany). Assessment of the final electrode position was carried out. *C-fos* and 5-HT expression were quantitatively assessed, using a Leica DMRB microscope and the Stereoinvestigator software (MFB Bioscience, USA). The total number of positive cells was estimated using Abercrombie's correction formula [14].

2.2. Statistics

Two or three way ANOVAs with repeated measurements was used (Statistica, Statsoft, USA) and main effects tested for Groups and Sessions. When appropriate, post-hoc analysis was performed using Student–Newman–Keuls test. Level of significance was set at $p < 0.05$. Results are expressed as means ± standard error of the mean (SEM).

3. Results

For immunohistochemical and behavioral analysis, a total of 16 animals out of initial 21 were included. One rat died following bilateral 6-OHDA injection and 4 were excluded due to electrode misplacement. Fig. 2 shows a schematic representation of bilateral electrode placement in the MFB of animals selected for the study.

3.1. Immunohistochemical analysis

Bilateral injection of 6-OHDA within the ventral tegmental area (VTA) led to a depletion of TH-positive cells and fibers in the mesolimbic/mesocortical projections and terminals in the MFB and the forebrain, confirming the loss of dopaminergic neurons and their projections. However, histological analysis showed variable degree of chemical lesion of the dopaminergic system probably due to local spread of 6-OHDA to areas adjacent of the VTA, such as the MFB and the substantia nigra pars compacta (Fig. 3A–F). Stereological assessment of TH-positive cell count showed significant decrease of TH+ cell numbers within the "VTA-lx group" compared to the non-lesioned controls, both in the VTA (VTA-lx 203 ± 22 vs Control 527 ± 24) and in the SNc (VTA-lx 194 ± 34, Control 394 ± 26), ($p < 0.001$) (Fig. 3G). This finding corroborates the TH+ fiber density assessment, which indicated a reduction in afferent areas of the midbrain dopaminergic projections in the lesioned animals, mainly in the NAC, but also in the dorsal striatum (respectively, 48% and 63% in comparison to the control), ($p < 0.001$) (Fig. 3H).

Depletion of the ascending dopaminergic projections also reduced activation of target areas of the MFB, translated as a decreased expression of the early gene *c-fos* both in the medial prefrontal cortex (VTA-lx 19 ± 5 vs Control 125 ± 31, $p = 0.004$) and in the NAC (VTA-lx 86 ± 21 vs Control 10 ± 2, $p = 0.002$) (Fig. 3G). Further, a significant decrease in serotonin expression in the dorsal raphe nucleus was also observed in the lesioned animals (VTA-lx 59 ± 17 vs Control 195 ± 23, $p < 0.001$) (Fig. 3G).

The VTA-lx animals presented variable degree of dopamine depletion, and in order to assess whether the extension of 6-OHDA lesion correlated with histological and behavior measures, animals were allocated into sub-groups. The 6-OHDA-lesioned rats were reclassified as "Severe VTA-lx", when the VTA TH-positive cell count was below 50%, or "Mild VTA-lx", when the loss was less than 50% compared to the control group (Fig. 3A–F). Indeed, further analysis showed strong decrement of *c-fos* activation in the PRL and NAC in "severely-lesioned" and "mildly-lesioned" animals in comparison to controls (Groups, $F(2,13) = 5.50$, $p = 0.03$ and 0.04, respectively) (Fig. 3I). Furthermore, 5-HT expression in the dorsal raphe nucleus following MFB-DBS was also dependent on the integrity of the dopaminergic system: the Severe VTA-lx rats had lower serotonin-positive immunolabeling in the DRN than the Mild VTA-lx group ($p = 0.02$) and controls ($p < 0.001$), (Groups $F(2,13) = 1.76$) (Fig. 3I).

3.2. Health status and behavioral assessment

Based on the histological findings, data for statistical analysis of the performance of the animals in the behavioral test sessions was processed taking into account the degree of dopaminergic depletion in the study groups, i.e. "Severe-VTA-lx", Mild-VTA-lx and Control. Bilateral injection of 6-OHDA into the VTA led to a significant drop of weight among the VTA-lx groups in comparison with the non-lesioned control group (Groups × Sessions $F(36, 185) = 3.07$, $p < 0.01$). Although this difference in body mass decreased at some point, the follow-up weight curve shows a clear impairment of the VTA-lx groups in gaining weight, so that even before stimulation started, a significant difference in body-weight between groups was present and remained until the end of the

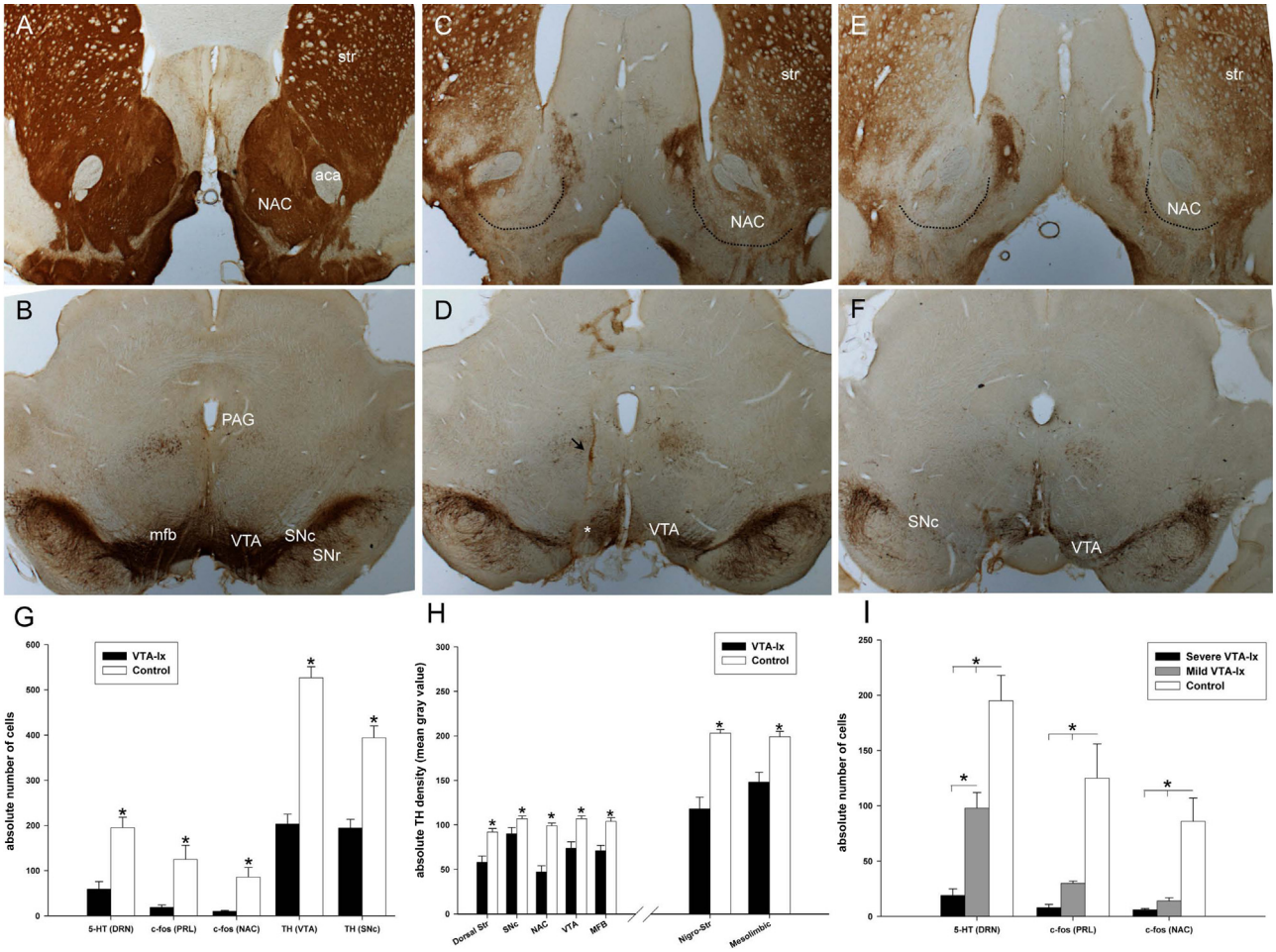

Fig. 3. (A–I) Histological assessment. (A–F) TH staining showing strong positive immunolabeling within the components of the nigrostriatal and mesolimbic systems: (A–B) control group (non-lesioned), (C–D) 6-OHDA VTA-Mild-lesioned animals, (E–F) severe lesioned group; (G) 6-OHDA VTA-injection led to a significant decrease of TH+ cells within the VTA itself, but also in the substantia nigra pars compacta, due to the spread of the toxin. Following MFB-DBS, the reduced number of ascending dopaminergic fibers towards the ventral striatum and forebrain gave rise to significantly less activation of these target regions, as shown by decreased *c-fos* expression. Depletion of DAergic fibers also led to less 5-HT expression in the dorsal raphe nucleus; (H) decreased number of dopaminergic cells in the VTA and SNc led to a reduction of TH+ fiber density in the ventral and dorsal striatum, respectively; (I) *c-fos* and 5-HT expression following MFB-DBS varied according to degree of dopamine depletion. NAC, nucleus accumbens; aca, anterior commissure; str, striatum; PAG, periaqueductal grey; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; VTA, ventral tegmental area; mfb, medial forebrain bundle; white *, lesioned VTA; arrow, injection path; black *, $p < 0.05$.

experiment. During the first days of MFB-DBS a discrete drop in weight was observed in all groups, which stabilized and recovered even under continuous stimulation. Stimulation-induced weight loss occurred in parallel with a subjective increment in motor activity and drop of food intake by 25%, during the initial 72 h of stimulation, in all groups (Sessions F (11,154) = 6.82, $p < 0.001$).

Affective status assessed via USV test revealed a significant group effect at the post-lesion/baseline session in both the High (F (2,13) = 3.76, $p < 0.05$) and the low frequency range (F (2,13) = 3.97, $p < 0.05$) ranges. A strong trend towards an increased number of 22 kHz calls (low) was observed among severely lesioned vs mildly lesioned animals in comparison with the controls ($p = 0.06$ and $p = 0.09$, respectively; Fig. 4A). A significative lower incidence of USVs in the 50 kHz range (High) could be observed in both VTA-lesioned groups compared to the controls (Severe-VTA-lx vs Control, $p < 0.05$; Mild-VTA-lx vs Control, $p < 0.05$). MFB-DBS reduced the occurrence of USVs in the 22 kHz range and increased the number of 50 kHz events in all groups ($p < 0.01$), indicating that in a relatively non-stressful environment, following stimulation, the profile of USVs were similar across groups (Fig. 4A). On the other hand, under stress, severe dopamine depletion precluded MFB-DBS from rescuing depressive-like phenotype as assessed in the FST (Fig. 4B; Groups × Session F (26,169) = 1.61, $p = 0.03$). Significantly increased immobility in the Severe-VTA-lx group was detected during the last 3 min of the FST in comparison with Mild-VTA-lx and Control groups ($p < 0.05$); however no difference was observed between mildly lesioned rats and controls ($p = 0.9$) (Fig. 4B). Furthermore, the SPT revealed a strong effect of both interventions in hedonic behavior (Fig. 4C; Groups, F (2,13) = 1.05, $p < 0,01$; Sessions, F (1,13) = 11.07, $p < 0.001$). Severe mesolimbic/mesocortical dopamine depletion led to a significant increased sucrose consumption compared with the Control ($p = 0.02$). DBS treatment led to an overall decrease of sucrose preference in comparison with the baseline assessment, and similar to the first testing, there was a positive correlation between the degree of VTA dopamine depletion and sucrose preference ($p < 0.05$). In the EPM, neither VTA lesion nor MFB-DBS produced any observable behavioral changes in the anxiety-like affective status (Fig. 4D).

4. Discussion

Since its first description by Olds and Milner in the 50s, electric stimulation of the MFB and associated pathways, mainly based on

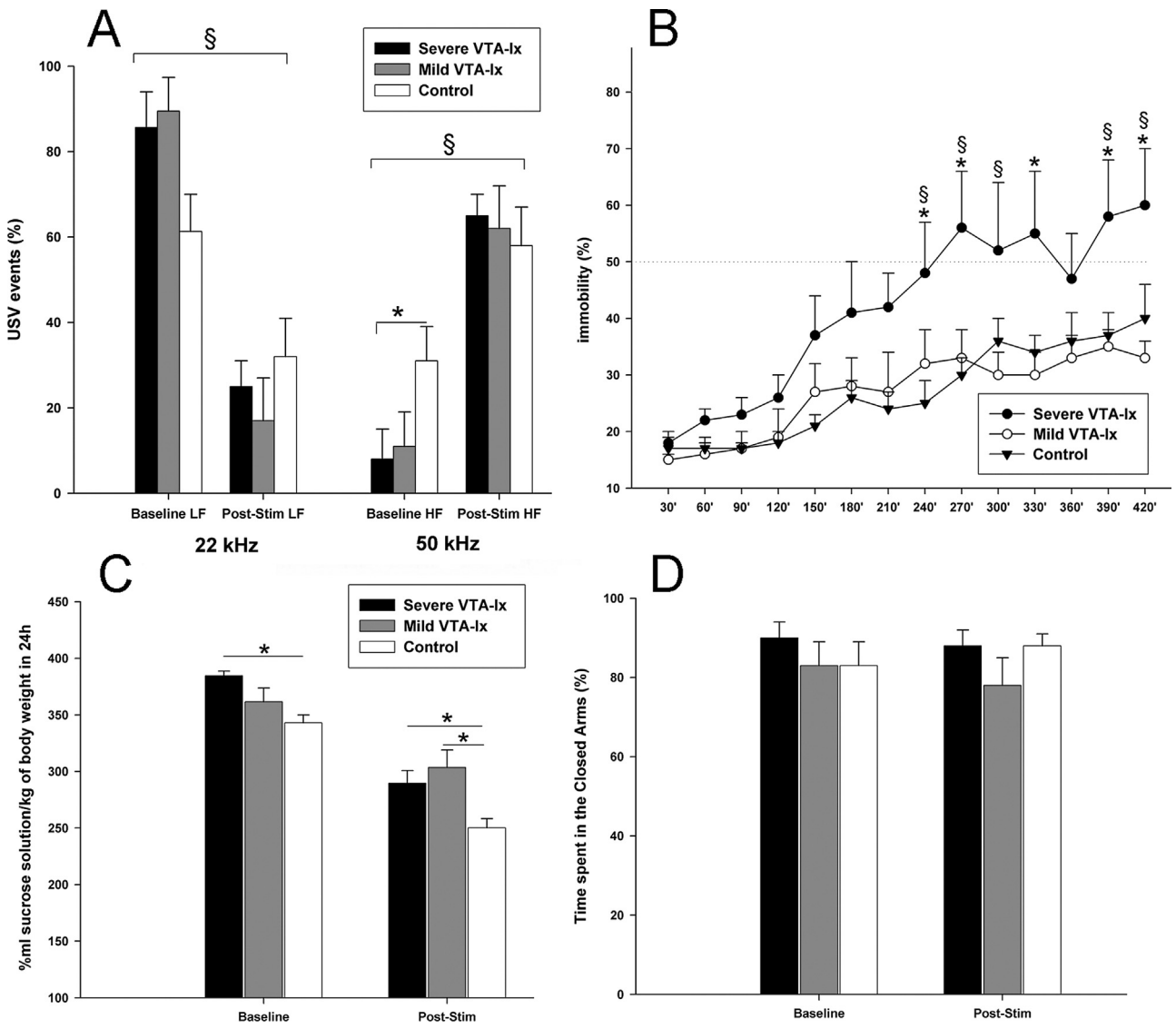

Fig. 4. (A–D) Behavioral tests. (A) In comparison to the controls, bilateral partial dopamine depletion in the VTA led to a significant increase of low frequency "negative affect" calls, as well as a decrease of the number of events in the high frequency range, i.e., reduction of 50 kHz "positive affect" calls. MFB-DBS induced recovery of this phenotype in both groups, with significant reduction of 22 kHz calls paralleled with increased percentage of high frequency calls. §: session effect (baseline × post-stim); *: groups effect ($p < 0.05$). (B) In the forced-swim test, data presented as percentage of immobility in time intervals of 30 s show that under stress severe dopamine depletion precludes MFB-DBS in rescuing depressive-like phenotype ($p < 0.05$). (C) Anhedonic-like behavior was evaluated by a sucrose preference test. A positive correlation between the degree of VTA dopamine depletion and sucrose preference was detected ($p < 0.05$). Despite of MFB-DBS, severe mesolimbic/mesocortical dopamine depletion led to significantly increased sucrose solution consumption in comparison with the Control group ($p = 0.02$). (D) Neither VTA lesion nor MFB-DBS correlated with anxiety-like behavioral changes as observed in the elevated plus maze test.

acute intracranial self-stimulation paradigm, has been applied in pre-clinical models of psychiatric disorders to investigate mechanisms of reward and addiction [3,25]. In line with data emerging from the field of affective neuroscience suggesting that the MFB sub-serves the SEEKING (motivation, drive, "wanting") behavior, the rationale for a direct stimulation of the human superolateral branch of the MFB (slMFB) in major depression was the observation that co-stimulation of the slMFB lead to hypomania in patients undergoing subthalamic nucleus DBS for advanced Parkinson's disease [10,12,26]. In a subsequent analysis, the MFB came into the focus as potential therapeutic target for therapy refractory depression since the previously stimulated target regions (ventral capsule/ventral striatum, nucleus accumbens, subgenual cingulate gyrus) appear to be connected in the MFB system [26–28].

Although groups have speculated about the antidepressant effects of MFB DBS in the light of rapid and sustained clinical responses [4,9], hypotheses concerning the mechanistic explanations for how the MFB's neuromodulation impacts on the network-model of depression are under debate [3,29]. Optogenetic methods appear to attribute a role in depression genesis to the reward circuitry and especially to the VTA [30,31]. In this respect lesioning the mesolimbic and mesocortical DA-projections ascending from the VTA should hypothetically lead to a depressive-like phenotype, but also prevent at some degree optimal activation of the reward-SEEKING system via neuromodulation of the MFB.

In the current experiment, behavioral assessments and stereological immunohistochemical analysis were employed to shed light on possible biological mechanisms of bilateral chronic continuous high-frequency stimulation of the MFB in rescuing a depressive phenotype. Our data show, that although upregulation of neural activity in MFB-downstream and upstream brain structures is dependent upon the integrity of ascending dopaminergic fibers, severe bilateral VTA dopamine depletion: (i) did not preclude MFB-

DBS in rescuing depressive-like phenotype as assessed via USV recordings; (ii) but prevented MFB neuromodulation – using clinically relevant stimulation parameters – of improving affective state in a stressful situation (FST); (iii) interestingly, led to an imbalance of affective response, favoring the "liking" rather than the "wanting" aspect of reward, as observed in the sucrose preference test (SPT). The data point towards the importance of dopamine's interplay with other neurotransmitters in the underlying mechanisms of MFB as a target for neuromodulation of the depression network, and challenges the concept of a linear relationship between dopamine levels and "liking" behavior.

USV paradigms have shown proof of principle in preclinical research for assessment of animal's affective state [17,32–34]. It has been shown that rats emit distinct types of USVs, which varies in frequency, depending on age, environmental factors and the subject's current state. Low frequency (LF) vocalizations (around 22 kHz) reflects a negative affective or aversive behavior, such as fear conditioning [35], first time handling [36] or following social isolation [37], whilst USVs in the high frequency (HF) range (50 kHz) correlate with pleasurable or rewarding experiences [17,32,33,38–40]. In our experiment, bilateral VTA dopamine depletion led to a decreased number of calls in the 50 kHz range and a higher number of aversive ultrasonic vocalizations compared to the controls. MFB-DBS, potentially by activating the SEEKING system [27,41], led to a significant increase in the number of 50 kHz calls with concomitant decrease of 20 kHz USVs, regardless of the integrity of the dopaminergic pathways. On the other hand, VTA severely lesioned animals presented stronger despair phenotype in the FST, despite of chronic MFB-DBS treatment, in comparison with mildly lesioned and non-lesioned control. This behavioral dissociation between the experimental sub-groups is a robust indication of the importance of the intactness of dopaminergic VTA-NAC/VTA-PRL projections in underlying mechanisms of MFB-DBS in modulating the mesolimbic/mesocortical circuitries in this condition. Further, neuromodulation of the MFB led to an increased expression of the early gene *c-fos* in the shell of the NAC and PRL, as well as of 5-HT in the dorsal part of the raphe nucleus. The expression of *c-fos* and 5-HT in key areas of the SEEKING system varied proportionally to the degree of bilateral dopaminergic cell depletion in the VTA which, in turn, seemed to condition the variable positive effect of MFB neuromodulation in rescuing depressive-like phenotype in different environmental/stress conditions. Decreased number of 5-HT positive cells in the DRN among "severe" dopamine-depleted rats might be interpreted as an effect of secondary neuronal degeneration due to the loss of their efferent targets in the VTA.

Anhedonia, a typical symptom in clinical depression and characterized by the decreased ability of experiencing pleasure in activities otherwise pleasurable, is typically assessed in animal models via a SPT protocol [22,30]. The hedonic component – "liking" – of the reward response is related to brain structures such as orbitofrontal cortex, anterior cingulate and medial portion of the nucleus accumbens shell, however mediated mainly by opioids, endocannabinoids and GABA-benzodiazepine neurotransmitter systems. On the other hand, dopamine is associated more with driving the "wanting" aspect of reward which is linked to motivation [10,42–44]. In our experiment, reduction of the dopaminergic input in the NAC shell might have led to an imbalance of this system, increasing *liking* behavior in the VTA lesioned subjects as observed in the SPT. This finding could have possible implications in future investigations and neurosurgical treatment of other diseases like eating disorders [44,45].

Translational studies using animal models of diseases have limitations and, therefore, must always be interpreted with caution [46]. It has been previously demonstrated that adult rats following complete bilateral MFB lesions, i.e., both mesocorticolimbic and nigrostriatal dopamine depletion, develop severe motor impairments and may not survive [47]. Here, one could initially not exclude that, although stereotactic 6-OHDA injection has targeted only A10 dopaminergic neurons (VTA), due to the spread of the toxin, nigrostriatal projections could also have been affected, leading to, for instance, increased immobility in the FST and therefore to biased analysis of behavioral outcome. However, there is evidence that contradicts this idea: firstly, apart from a lesion-induced difference in body-weight dynamics (also present in other rodent models of depression [23,48]), aphagia, adipsia or motor impairment could not be observed across groups. Secondly, by the time-point when baseline behavior tests were performed (at least 1 month following 6-OHDA injection), no differences in performance in the FST were observed across groups. Considering that the timeframe between lesion and baseline behavior tests was long enough for a complete stabilization of lesion-induced deficits [49,50], it is not likely that undesired nigrostriatal lesion could have interfered in behavioral outcome. Furthermore, histological analysis revealed low degree of nigrostriatal lesion in comparison with non-lesioned controls, suggesting that basal extracellular DA levels in the dorsolateral striatum might not have been affected [49].

In summary, the data suggests that (i) bilateral DA lesions of the VTA affecting the ascending mesolimbic and mesocortical pathways lead to the production of a depressive phenotype in rats as shown with the USV and FST paradigms. This result is in concordance with previous publications suggesting that a dysfunctional reward system is an important contributor to depression genesis; (ii) MFB-DBS reduced the depressive-like phenotype and lead to activation of distant structures involved in the neurocircuitry of depression; (iii) integrity of midbrain dopaminergic cell projections play an important role in the dynamics of the SEEKING system and seems to directly correlate with the degree of neural activation in the NAC and PRL; (iv) rescuing of depressive-like phenotype by MFB-DBS might not depend exclusively upon dopaminergic transmission [51], which reinforces the concept of MFB-DBS as neuromodulation not of a single target, but of a whole network of circuits [3,4,27]. Further investigations are essential to highlight the role of dopamine and other transmitters in the therapeutic mechanisms mediated by MFB-DBS.

5. Conclusion

Bilateral chronic continuous MFB-DBS results in temporary increase in exploration, which could explain the initial weight loss and decreased food intake observed [15]. The capacity of MFB-DBS to reverse depressive-like phenotype seemed to be a factor of the integrity of the ascending dopaminergic fibers and the stress associated with the behavioral task. Continued investigation on the network of depression using neuromodulation platforms in animal models is crucial to further elucidate mechanisms of the disease and of DBS in the treatment of neuropsychiatric disorders.

Disclosure

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