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Bilateral subdiaphragmatic vagotomy modulates the peripheral met-enkephalin and striatal monoamine responses to peripheral inflammation in rat

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In the central nervous system, long-term effects of a vagotomy include disturbance of monoaminergic activity of the limbic system. Since low vagal activity is observed in major depression and autism spectrum disorder, the study aimed to determine whether animals fully recovered after subdiaphragmatic vagotomy demonstrates neurochemical indicators of altered well-being and social component of sickness behavior. Bilateral vagotomy or sham surgery was performed in adult rats. After one month of recovery, rats were challenged with lipopolysaccharide or vehicle to determine the role of central signaling upon sickness. Striatal monoamines and met-enkephalin concentrations were evaluated using HPLC and RIA methods. We also defined a concentration of immune-derived plasma met-enkephalin to establish a long-term effect of vagotomy on peripheral analgesic mechanisms. The data indicate that 30 days after vagotomy procedure, striatal dopaminergic, serotoninergic, and enkephalinergic neurochemistry was altered, both under physiological and inflammatory conditions. Vagotomy prevented inflammation-induced increases of plasma met-enkephalin – an opioid analgesic. Our data suggest that in a long perspective, vagotomized rats may be more sensitive to pain and social stimuli during peripheral inflammation.

Key words: striatum, subdiaphragmatic vagotomy, met-enkephalin, inflammation, monoamines, pain, social withdrawal, sickness behavior

INTRODUCTION

It is well-known that peripheral immune challenges influence brain neurochemistry (Dunn, 2006) and vagus nerve fibers constitute one of the routes mediating this interaction (Reardon et al., 2018; Schiller et al., 2021). The vagus nerve preserves local pro- and anti-inflammatory balance *via* its efferent signaling capabilities (Zila et al., 2017; Tanaka et al., 2019) but also participates in immune-to-central nervous system (CNS) communication. Immune signals from the periphery reaching the brain through vagal sensory fibers trigger the cholinergic anti-inflammatory pathway which is a part of the inflammatory reflex (Abe and Inoue, 2018), the hypothalamic-pituitary-adrenal stress axis (HPA) (Herman, 2018), and sickness behavior (McCusker and Kelley, 2013).

Knowledge about the immunological functions of the vagus nerve comes mainly from studies that use different versions of vagotomy (e.g., mechanical or chemical damaging of vagal fibers) followed by immunogen injections. Vagotomy's effectiveness in controlling inflammatory processes is evidenced by its ability to inhibit central and peripheral effects of immunogen (Table 1).

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Table 1. Literature data about the influence of vagotomy (VG) on various biochemical, histological and behavioral parameters in rat. Part A describes influence of VG on experimental i.p. inflammation. Part B describes effects of VG under non-septic conditions. Part C describes VG-specific changes not induced during peripheral inflammation in sham rats.

A: VG-caused disruption of physiological immunogen effects			
Type of i.p. injected immunogen	Immunogen effect in sham rats	VG effect on response to immunogen	Time of recovery after VG procedure
IL-1β	Decreased NE in HPT	Blocked	7-14 days[1]
	Increased NE in medial HPT	Blocked	10 days[2]
	Increased NE in PVN	Blocked	4 weeks[3]
	Increased plasma corticosterone	Reduction ca. 50%,[1] Partially blocked[2]	7-14 days[1], 10 days[2]
	Increased plasma ACTH	Partially blocked	10 days[2]
	Shivering	Blocked	10 days[2]
LPS	Increased cFOS in SON	Blocked	7 days[4]
	Increased cFOS in PVN	Blocked	7 days[4], 33 days[5]
	Increased Ucn2 mRNA in PVN and adrenal medulla	Blocked	33 days[5]
	Increased liver NO content	Blocked	hours[6]
	Increased number of activated cytotoxic T-cells in blood	Blocked	hours[7]
B: VG-induced changes in basal rat physiology			
Increased level of MDA and NO in brain and liver			hours[6]
Decreased level of reduced form of GSH in brain and liver			hours[6]
Increased serum levels of ALT, AST and ALP			hours[6]
Decrease in basal: • number of total lymphocytes and B-lymphocytes in blood • number of granulocytes and DC-cells in spleen			hours[7]
C: Inflammation-induced physiological changes specific to vagotomized animals			
Decrease in cell number during inflammation: • NK-cell in blood • total lymphocytes, T-lymphocytes, T-helper cells, cytotoxic T-cells, activated cytotoxic T-cells, B-lymphocytes, DC-cells, NK-cells in spleen			hours[7]

ACTH – adrenocorticotropic hormone, ALP – alkaline phosphatase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GSH – glutathione, HPT – hypothalamus, IL-1β – Interleukin 1β, LPS – lipopolysaccharide, NE – norepinephrine, NO – nitric oxide, MDA – malondialdehyde, PVN – paraventricular nucleus of hypothalamus, SON – supraoptic nucleus, Ucn2 – urocortin 2. References: 1. Fleshner et al. 1995, 2. Wieczorek and Dunn 2006, 3. Ishizuka et al. 1997, 4. Wan et al. 1994, 5. Tillinger and Mravec 2021, 6. Abdel-Salam et al. 2013, 7. Mihaylova et al. 2014.

One of the key variables affecting research on vagus-mediated immune-CNS communication is the recovery time between vagotomy and induction of inflammation. Ghia et al. (2007) demonstrated that shortly after vagotomy, the immune response to the experimental-induced disease was intensified, but along with prolonged recovery, it returned to normal. Nevertheless, after 2 months of recovery, vagotomy engenders some relatively stable changes to the immune system activity, like the cytokine profile in inflamed tissue. Mitsui et al. (2014), showed that even without inflammation, the cytokine milieu of vagally denervated jejunum was altered in a manner dependent on the recovery period after vagotomy, and increased TNF-α and MCP-1 were not observed until 20 days after surgery. Both studies describe the time-dependent effect

of vagotomy on the general intensity of inflammatory processes and indicate that an organism may develop immunological alterations that counteract the lack of vagal anti-inflammatory activity.

Our previous research showed that, following a 30-day recovery period, vagotomy disturbs the activity of brain dopaminergic and serotoninergic systems and may affect rat behavior (Kobrzycka et al., 2019). While analyzing standard behavioral parameters of the elevated plus maze (EPM) test like locomotor activity or anxiety, we additionally noticed group-specific closed-arm preferences. Saline-injected sham animals had no preference for the left *vs.* right closed arm and spent similar time in both. Sham animals with ongoing inflammation and vagotomized non-inflamed animals spent more time in the left closed arm. In contrast, the vagotomized animals with ongoing inflammation preferred the opposite, right closed arm. In our EPM test, we did not expect any social observations, and such behavior was not observed in sick sham individuals. However, we believe that this might have been observed in the case of vagotomized rats with ongoing inflammation. Especially, that Ghizoni et al. (2006), using a modified EPM test protocol showed that vagotomy itself may indeed weaken emotional learning.

We investigated striatal neurotransmission as a potential explanation for the observed behavioral phenomenon as the striatum regulates social behavior. It was shown that activity of the nucleus accumbens (NA, one of the striatal nuclei) reflects the motivation to obtain social reward and to avoid social punishment (Kohls et al., 2013) and during inflammation, positive social stimuli increase the activity of the ventral striatum which includes NA (Báez-Mendoza et al., 2013; Inagaki et al., 2015; Muscatell et al., 2016). Moieni and Eisenberger (2018) summarized that the social aspect of the sickness behavior is not simply affected by the suffering of the sick animal, but rather mediated by increased neural sensitivity of the reward system to both positive and negative social stimuli. Hence, we decided to test if vagotomy influences striatal monoaminergic systems and met-enkephalin synthesis during the peripheral immune challenge.

At the same time, we hypothesize that altered social behavior of vagotomized animals might result from worsened mood caused by increased pain feelings during inflammation. Hence we measured plasma met-enkephalin – a major analgesic compound at the early stages of inflammation.

To ensure that we study the mechanism(s) that compensate for vagal dysfunction, we performed experiments 30 days after the surgery procedure at the early stages of the inflammatory response (2 h after LPS injection), when in normal conditions the vagus nerve plays the main role in immune-CNS communication.

METHODS

Animals

The study was performed on 3-month-old male Wistar rats (n=60, 300 g ± 25 g, at the beginning of the experiment). Animals were individually housed in breeding cages under artificial lighting conditions (a 12-h day-night cycle, light on at 7:00 AM) with free access to water and feed (Purina granules). The temperature in the animal facility was set at 21-22°C and humidity at 60-65%. Before the experiment, rats were habituated for 7 days to the conditions in the animal facility. All applied experimental procedures were carried out with the approval of the Local Ethical Commission, 73/ŁB582/2012.

Experiment

Animals were divided into two main groups: sham-operated (SH, n=30) and bilateral subdiaphragmatically vagotomized (VG, n=30). All animals were subjected to surgery under general anesthesia (i.p., Innovar plus, 6 µl/g body weight) and local anesthesia (subcutaneous, 2% lidocaine solution, with noradrenaline 0.5 ml/animal). In the VG group, small fragments of gastrointestinal and hepatic branches of the nerve were cut just below the diaphragm. The sham procedure was performed in an analogous way to vagotomy, with exception of cutting the nerves. A full description of both procedures can be found in Kobrzycka et al. (2019). To prevent postoperative infection and to facilitate wound healing, an antibacterial agent (Alu Spray, V.M.D.) was applied to the operation site. Following surgeries, animals were returned to their cages, and after a 30-days recovery period, animals from both groups were randomly assigned to receive i.p. injection of saline (0.9% NaCl, 100 µl) or LPS (10 µg E. coli 026: B6 in 100 µl 0.9% NaCl, 100 µl) and 120 min later, animals were euthanized (Supplementary Fig. 1).

Samples preparation procedures

For the ELISA test of proinflammatory cytokines, plasma was obtained by centrifuging the trunk blood collected on EDTA (1 ml/100 µl of Na_2EDTA) at 4000 rpm

for 10 min at 4°C. Obtained plasma was frozen and stored at -80°C until future analysis.

Because of known anatomical and functional lateralization (Guarneri et al., 1985; Larisch et al., 1998; Capper-Loup et al., 2009), left and right striatum was analyzed separately. Left and right striatum from each animal were isolated immediately after decapitation, according to the Paxinos and Watson (1998) stereotaxic atlas (AP: -1.4 to 2.3), then were immediately weighed and frozen on dry ice. Afterward, all samples were stored at -80°C till further analysis.

HPLC of monoamines and ELISA test

Striatum samples were thawed, homogenized with an ultrasonic homogenizer (BioBlock Scientific) in 150 µl of homogenization solution (0.4 mM $Na_2S_2O_5$, 0.6 mM $HClO_4$), centrifuged at 12,000 rpm for 15 min at 4°C. The collected supernatant was transferred to chromatographic tubes and analyzed. The concentration of the main monoamines: noradrenaline (NE), serotonin (5HT), and dopamine (DA), as well as their metabolites: 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) was determined in collected brain samples with the RP-HPLC-ED isocratic method. An Agilent 1100 chromatographic system with Waters Spherisorb ODS-1 RP C-18 chromatographic column (4.6 × 250 mm) preceded by a Zorbax SB-C18 pre-column (4.6 × 12.5 mm) was used. Conditions during analysis were set as follows: column temperature 35°C, mobile phase flow 1 ml/min, the potential of the glassy carbon working electrode relative to the Ag/AgCl reference electrode +0.65 V. The mobile phase consisted of a phosphate buffer (pH 3.4) containing: 0.15 M $NaH_2PO_4 \times H_2O$, 0.1 M Na_2EDTA, 0.5 mM Na_2OSA, 0.5 mM LiCl, with methanol (10%). The chromatographic data were analyzed using CHEMSTATION, REVISION-B.03.02 software (Agilent).

Using ELISA test, IL-1β concentration was established as a control variable for confirming the ongoing i.p. inflammation. Plasma IL-1β concentration was determined using the Rat IL-1β ELISA Kit from Diaclone (cat. no. 670.040), according to the manufacturer's instructions.

Native met-enkephalin concentration

Striatum samples were thawed, homogenized in 0.5 ml of 0.9% NaCl and centrifuged at 5000 rpm for 20 min at 4°C. The supernatant was used for RIA analysis. Native met-enkephalin concentration in the striatum and plasma was estimated by the radioimmunoassay method of Pierzchała and Van Loon (1990). Briefly, native enkephalin from plasma or striatum supernatant was purified on Porapak Q columns (Waters, 100-120 mesh) in 2 ml of absolute ethanol, lyophilized and assayed after reconstitution in 100 µl of 0.06 M phosphate buffer (pH 6.5, 0.2% bovine serum albumin, 0.002% sodium azide). The assay entailed the addition of 50 µl of antiserum (rabbit, 1:10000) and 50 µl of 125I-Met-enkephalin (~1500 cpm) and incubation at 4°C for 24 h. Antibodies-bound and free enkephalins were separated after 24 h by the addition of 50 µl of rabbit γ-globulin (1%) and incubation for 30 min at 4°C, followed by addition of 250 µl of 25% polyethylene glycol (PEG 8000), incubation for 30 min and finally, centrifugation (2000 × g, 4°C, 20 min). The supernatants were discarded, and the pellets were counted in a γ-counter (Wizard).

Statistical analysis

The experiment involves two independent variables (surgery and injection type), which means that usage of the two-way ANOVA would be appropriate. Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Levene test). Because some data did not meet the parametric assumptions, we decided to use single comparisons tests with Bonferroni correction for multiple comparisons. Because of the number of comparisons, p-values lower than 0.0125 ($p<0.05/4$) were considered to be statistically significant. Data meeting the assumptions of the parametric test were analyzed with Student t-tests; others were tested using the non-parametric Mann-Whitney U test. Analogous to the analysis performed in our previous work (Kobrzycka et al., 2019), the following two-tailed sub-hypotheses were tested and displayed on the figures: SH+NaCl i.p. *vs.* VG+NaCl, SH+NaCl i.p. *vs.* SH+LPS, VG+NaCl i.p. *vs.* VG+LPS, SH+LPS i.p. *vs.* VG+LPS. All statistical analyses were performed using the STATISTICA software, version 13.3 (TIBCO Software Inc., 2017).

RESULTS

In the proposed experiment (Fig. 1), vagotomy did not affect IL-1β under non-septic conditions (SH NaCl *vs.* VG NaCl U=94, p=0.509). Inflammatory responses measured by the increase of amount IL-1β (a pro-inflammatory cytokine) in plasma was observed in both groups injected with LPS; sham-operated (SH NaCl *vs.*

Table 2. Results of statistical analysis.

		SH NaCl *vs.* SH LPS	VG NaCl *vs.* VG LPS	SH NaCl *vs.* VG NaCl	SH LPS *vs.* VG LPS
Plasma	IL-1β	**U=16, p<0.001**	**U=39, p<0.001**	U=94, p=0.509	U=73, p=0.110
	Met-enkephalin	**U=1, p=0.002**	U=115, p=0.694	U=91, p=1.000	**U=0, p<0.001**
Right striatum	Met-enkephalin	***U=2, p=0.038***	U=7, p=0.352	U=2, p=0.114	U=13, p=0.485
	MHPG/NE	U=10, p=0.762	U=21, p=0.529	U=11, p=0.914	U=26, p=0.955
	5HIAA/5HT	U=0, p=0.017	**U=0, p<0.001**	U=17.5, p=0.142	**U=0, p=0.012**
	DOPAC/DA	**U=0, p=0.009**	U=33.5, p=0.815	**U=0, p<0.001**	**U=0, p=0.003**
	HVA/DA	U=12, p=0.788	U=12, p=0.234	U=19, p=0.142	U=5, p=0.171
Left striatum	Met-enkephalin	U=1, p=0.200	**U=0, p=0.002**	***U=0, p=0.017***	U=7, p=1.000
	MHPG/NE	t_7=1,07, p=0.320	t_{15}=0.68, p=0.51	t_{12}=0.97, p=0.352	t_{10}=-0.15, p=0.880
	5HIAA/5HT	***t_9=-2.53, p=0.032***	t_{12}=-1.30, p=0.217	t_{10}=-1.16, p=0.273	t_{11}=-1.01, p=0.336
	DOPAC/DA	U=12, p=0.662	U=19, p=0.573	U=22, p=0.852	U=5, p=0.082
	HVA/DA	U=10, p=0.527	U=15, p=0.152	U=21, p=0.463	U=7, p=0.230

Statistically significant p-value was 0.0125 (Bonferroni correction), p-value between 0.05 and 0.0126 was considered as a not-significant tendencies. Significant results are bolded and underline. Tendencies are bolded and cursive. SH – sham surgery, VG – subdiaphragmatic vagotomy, NaCl – i.p. saline injection, LPS – i.p. lipopolysaccharide injection. U – data tested with U Mann-Whitney test, t – data tested with Student t-test.

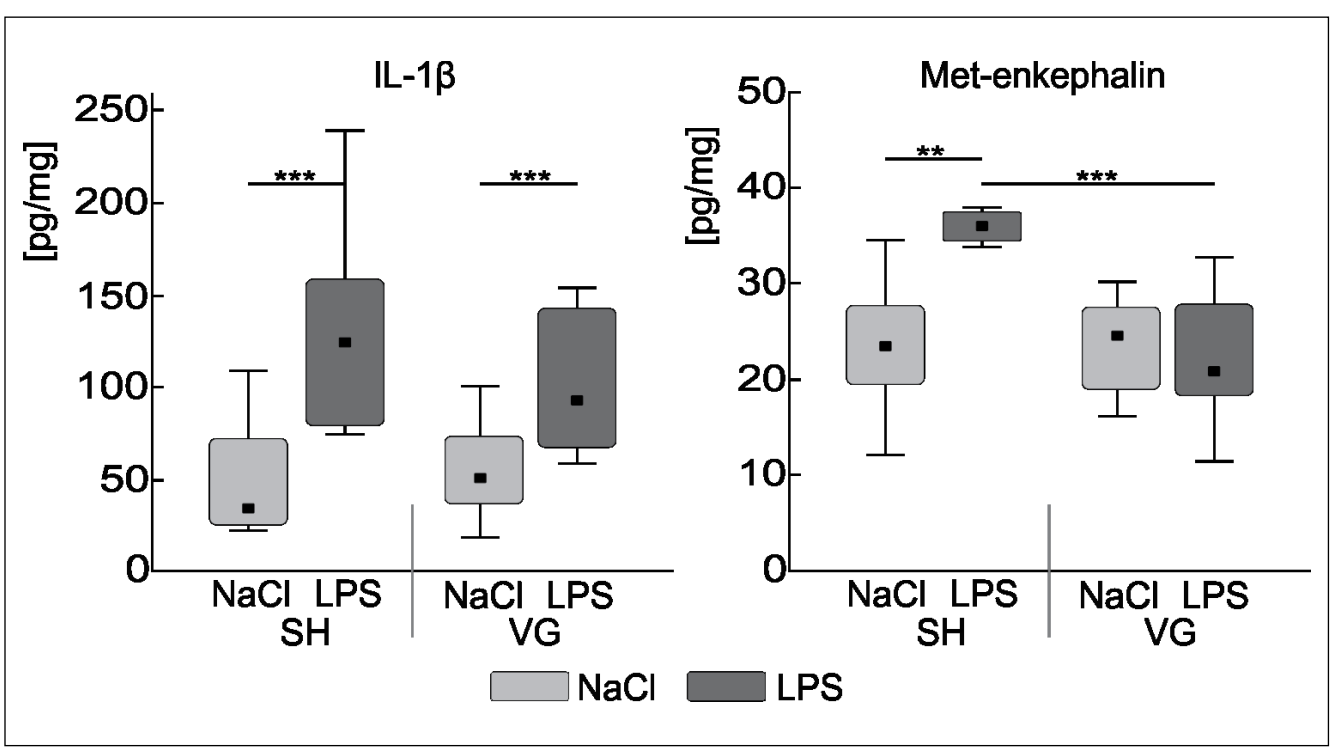


Fig. 1. Plasma concentration of IL-1β and met-enkephalin. SH – sham surgery, VG – subdiaphragmatic vagotomy, NaCl – i.p. saline injection, LPS – i.p. lipopolysaccharide injection, black square – median, box – quartile range, whisker – min-max range, ** – p-value < 0.0125, *** – p-value < 0.001.

SH LPS U=16, p<0.001) and vagotomized (VG NaCl *vs.* VG LPS U=39, p=0.001). LPS-induced increase of plasma IL-1β concentration was similar in both groups (SH LPS *vs.* VG LPS U=73, p=0.110).

Vagotomy did not affect plasma met-enkephalin concentration in non-inflammatory conditions (SH NaCl *vs.* VG NaCl U=91, p=1.000). However, it abrogated the increase of plasma met-enkephalin concentration after LPS injection (SH LPS *vs.* VG LPS U=0, p<0.001) – in vagotomized group, there was no LPS-induced increase of plasma met-enkephalin concentration (VG NaCl *vs.* VG LPS U=115, p=0.694), as it was observed in the sham group (SH NaCl *vs.* SH LPS U=1, p=0.002).

Met-enkephalin concentration in response to the surgical and LPS manipulations differed in the left and right striatum (Fig. 2). In the right striatum, vagotomy did not affect met-enkephalin concentration (SH NaCl *vs.* VG NaCl U=2, p=0.114). LPS injection has almost no effect on met-enkephalin; in the sham group, we observed a non-significant tendency to increase met-enkephalin concentration after LPS injection (SH NaCl *vs.* SH LPS U=2, p=0.038), however, such effect was not observed in the vagotomized group (VG NaCl *vs.* VG LPS U=7, p=0.352) and finally, there were no significant differences between groups (SH LPS *vs.* VG LPS U=13, p=0.485). In the left striatum vagotomy slightly, but no significantly, increased met-enkephalin concentration in non-inflammatory conditions (SH NaCl *vs.* VG NaCl U=0, p=0.017). In response to LPS injection, a significant increase of met-enkephalin concentration was observed in the vagotomized (VG NaCl *vs.* VG LPS U=0, p=0.002) but no sham group (SH NaCl *vs.* SH LPS U=1, p=0.200).

Monoaminergic neurotransmission in the striatum was also asymmetrically affected by vagotomy (Fig. 3). Experimental procedures did not affect any of the tested utilization indexes (UI) of monoaminergic neurotransmitters in the left striatum. Vagotomy did not affect parameters neither under non-inflammatory conditions (SH NaCl *vs.* VG NaCl, MHPG/NE t_{12}=0.97, p=0.352, 5HIAA/5HT t_{10}=-1.16, p=0.273, DOPAC/DA U=22, p=0.852, HVA/DA U=21, p=0.463) nor after LPS injection (SH LPS *vs.* VG LPS, MHPG/NE t_{10}=-0.15, p=0.880, 5HIAA/5HT t_{11}=-1.01, p=0.336, DOPAC/DA U=5, p=0.082, HVA/DA U=7, p=0.230). In the right striatum of vagotomized animals, significant changes of DOPAC/DA UI was found versus sham-operated animals; in the sham group, DOPAC/DA UI decrease after LPS injection (SH NaCl *vs.* SH LPS U=0, p=0.009) while such effect was not in vagotomized group observed (VG NaCl *vs.* VG LPS U=33.5, p=0.815) because of already increased DOPAC/DA UI in both no-inflammatory (SH NaCl *vs.* VG NaCl U=0, p<0.001) and septic conditions (SH LPS *vs.* VG LPS U=0, p=0.003). Vagotomy did not affect 5HIAA/5HT UI

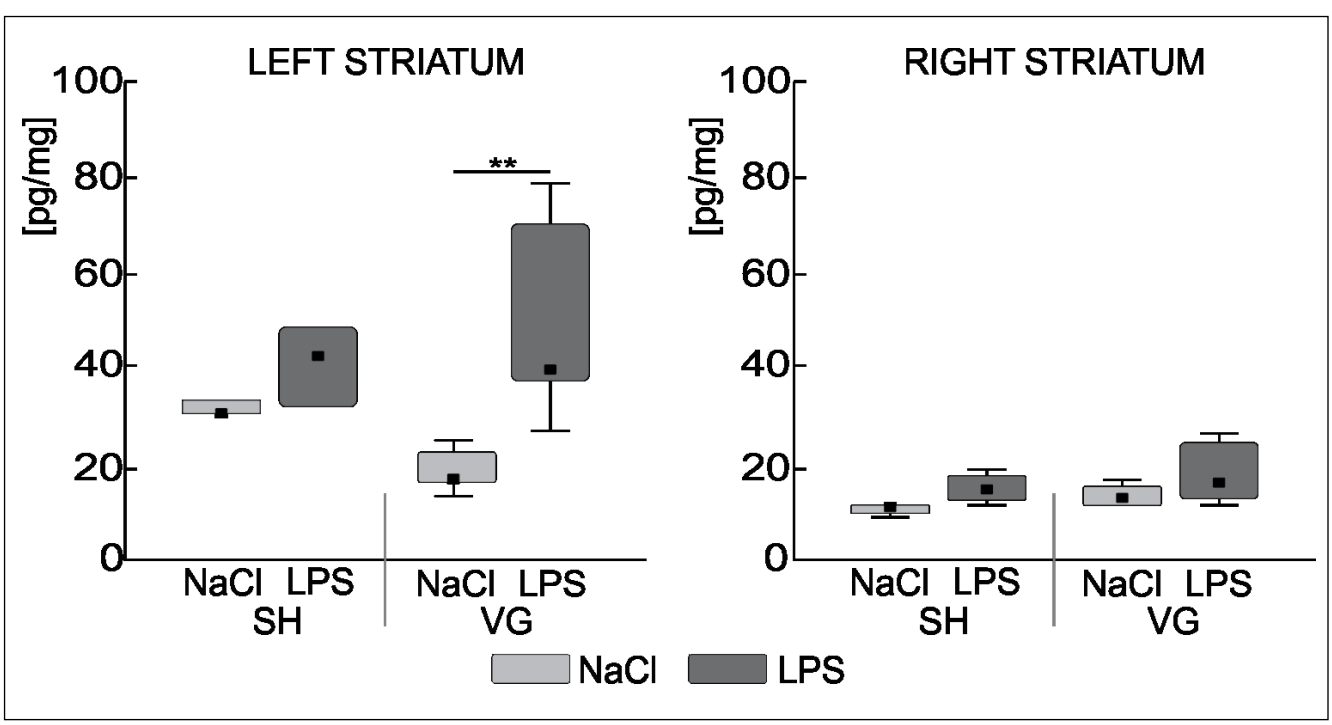


Fig. 2. Striatal concentration of met-enkephalin. SH – sham surgery, VG – subdiaphragmatic vagotomy, NaCl – i.p. saline injection, LPS – i.p. lipopolysaccharide injection, black square – median, box – quartile range, whisker – min-max range, ** – p-value < 0.0125, *** – p-value < 0.001.

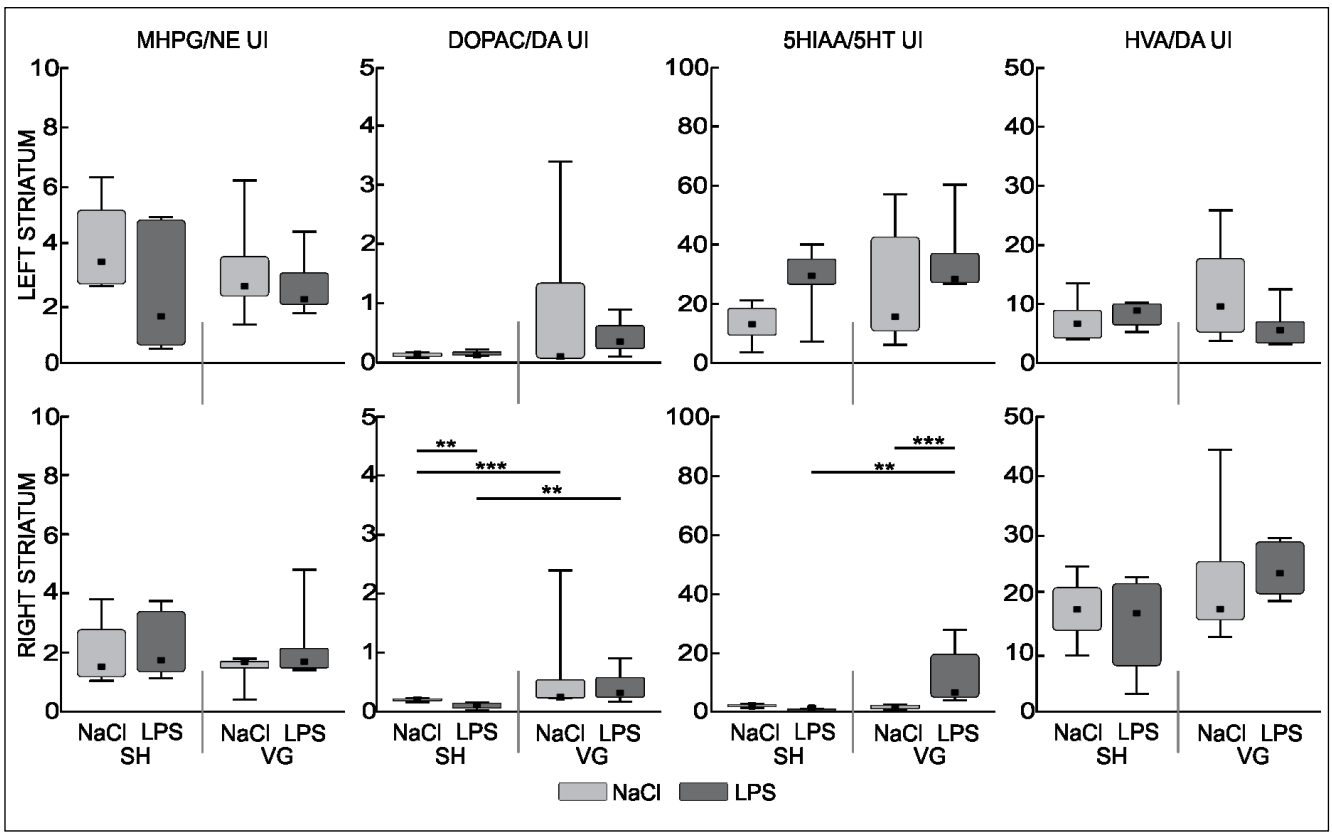


Fig. 3. Utilization indexes of main monoamines neurotransmitters in left and right striatum. SH – sham surgery, VG – subdiaphragmatic vagotomy, NaCl – i.p. saline injection, LPS – i.p. lipopolysaccharide injection, black square – median, box – quartile range, whisker – min-max range, ** – p-value < 0.0125, *** – p-value < 0.001.

under non-inflammatory conditions (SH NaCl *vs.* VG NaCl U=17.5, p=0.142). However, in the vagotomized group, 5HIAA/5HT UI significantly increased after LPS injection (VG NaCl *vs.* VG LPS U=0, p<0.001) and its level was significantly higher than in a similar sham group (SH LPS *vs.* VG LPS U=0, p=0.012) where LPS-induced non-significant tendency to decrease of 5HIAA/5HT UI (SH NaCl *vs.* SH LPS U=0, p=0.017). MHPG/NE and HVA/DA UI was unaltered, regardless of the experimental conditions (SH NaCl *vs.* VG NaCl, MHPG/NE U=11, p=0.914, HVA/DA U=19, p=0.142; SH LPS *vs.* VG LPS, MHPG/NE U=26, p=0.955, HVA/DA U=5, p=0.171).

DISCUSSION

Vagotomy alters striatal neurotransmission

In the present study, we found that vagotomy significantly increased the DOPAC/DA ratio in the right striatum in control and inflammatory conditions without affecting the HVA/DA ratio. In rat brains, DOPAC and HVA are the main two metabolites of dopamine (DA) (Meiser et al., 2013). Because of COMT enzyme location, HVA is formed extraneuronal (Elsworth et al., 1997), and HVA/DA ratio we interpret as a marker of dopaminergic signaling between cells. DOPAC can be formed both intra- and extraneuronal (Elsworth et al., 1997), however, the vast majority of the DOPAC origin from a recently-synthesized pool of DA (Soares da Silva and Garrett, 1990) deaminated by MAO_A enzyme located in the presynaptic neuron (Cho et al., 2021, Garrett and Soares da Silva, 1990). This is why DOPAC/DA ratio we interpret as a marker of presynaptic metabolism of DA.

Our results indicate that in vagotomized animals, DA metabolism inside neuron (DOPAC/DA ratio) increase. Unchanged intercellular dopaminergic signaling (HVA/DA ratio) indicates that DA neurotransmission in the striatum is attenuated after vagotomy. Such a conclusion is supported by the fact that DA release in the striatum depends on direct neurotensin (NT) and cholecystokinin (CCK) projection from nucleus tractus solitarii (NTS, nucleus with tonic sensory input from vagus nerve) (Wang et al., 1992). NT throughout NT_{S1} receptors (Widerlöv et al., 1982; Okuma et al., 1983; Hétier et al., 1988; Quirion et al., 1992; Nolan et al., 2020) and

CCK throughout the interaction of CCK_B receptor with D_2 receptor (Tanganelli et al., 2001) on presynaptic part of dopaminergic neurons projecting from the ventral tegmental area (VTA) and substantia nigra (SN), allows releasing DA in striatum. We concluded that when the vagus nerve is damaged or its activity is inefficient, the mechanisms regulating DA release in the striatum do not work properly and lead to an observed increase in the intracellular metabolism of DA. Moreover, in such conditions, direct serotoninergic projection from raphe nuclei (RN) becomes strengthened (Van de Kar and Lorens, 1979; Berger et al., 1985; McQuade and Sharp, 1997; Maeda et al, 2003; Waselus et al, 2006) and can partially take over the function of disturbed dopaminergic neurotransmission (Stotz et al., 1993; Maeda et al., 2005; Gagnon et al., 2016; Stemick et al., 2020). In fact, our results show an increase in serotonin metabolism (5HIAA/5HT ratio) in the right striatum of vagotomized animals under inflammatory conditions, and as shown by Karstaedt et al. (1994) an increased striatal 5HIAA/5HT ratio, can be an effect of disturbed DA neurotransmission (Fig. 4A).

Such conditions favor an intensification of met-enkephalin synthesis in the striatum. Long-term deficiency of the dopaminergic signal in the striatum may increase the synthesis of striatal met-enkephalin and serotonin $5HT_{1B}$ receptors (George and Kertesz, 1987; Kowalski and Giraud, 1993; Manier et al., 1991; Padovan-Neto et al., 2021). Also, serotonin stimulates striatal neurons to met-enkephalin synthesis (Walker et al., 1996; Mijnster et al., 1997; Padovan-Neto et al., 2021). Our results shown that met-enkephalin concentration increased in the striatum of vagotomized animals. However, while significant monoaminergic alterations were observed in the right striatum, a met-enkephalin significant increase was observed in the left one. Such an effect may be a consequence of anatomical and functional lateralization of the striatum (Fig. 4B). The right and left striatum of the rat differ in neuron density (Meitzen et al., 2011), dopaminergic metabolism (Thiel and Schwarting, 2001), and DA receptor (D_1 and D_2) density and affinity (Schneider et al., 1982; Franco et al., 2016).

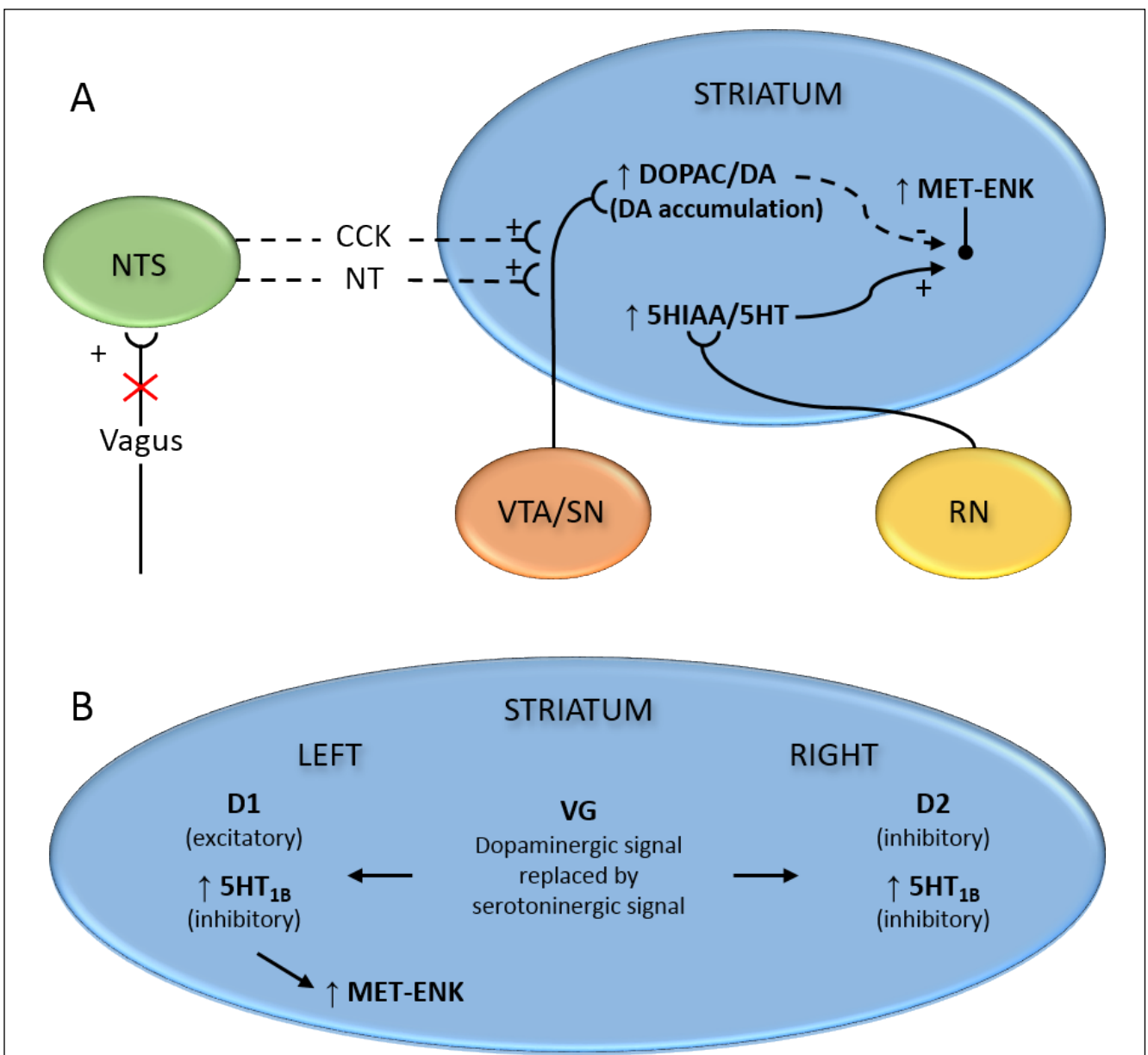
Striatal-managed natural side preferences or sensitization of the reward system

We set out to examine the influence of vagotomy on striatal neurotransmission given our previous observations from the EPM test presented in detail in Kobrzycka et al. (2019).

Thiel and Schwarting (2001) showed that striatal DOPAC/DA lateralization is involved in the side preference of thigmotactic scanning behavior – according to their results, we should expect that the saline-treated vagotomized group, which preferred the left arm of the maze, will also have an increased DOPAC/DA ratio in the left striatum and, similarly, the vagotomized group with ongoing peripheral inflammation, preferring the right arm of the maze, will have a significantly increased DOPAC/DA ratio in the right striatum. Data presented here show that both vagotomized groups have significantly increased DOPAC/DA ratio in the right striatum. Thus, the arm preference observed in our previous experiment may depend on a specific inflammation course in the vagotomized group, rather than a natural preference for the right or left side.

We hypothesize that observed in the previous experiment arm preference in the EPM arena may be linked with the social aspect of sickness behavior. Sick rats can seek companionship and support from familiars (Yee and Prendergast, 2010; 2012). On the other hand, social withdrawal is considered a typical sickness behavior not only limiting the risk of spreading the disease but also promoting rest and saving energy for fighting the infection (Hart, 1988; Dantzer, 2001). In natural conditions, social species may show social avoidance of sick animals by other members of the group or self-isolation of sick individuals (Loehle, 1995). Neuro-immune control of the behavior is linked with striatal activity (Rivera-Aguilar et al., 2008; Engler et al., 2009; Inagaki et al., 2015; Ben-Shaanan et al., 2016) and central dopaminergic neurotransmission (Eisenberger et al., 2010, Draper et al., 2018; Kopec et al., 2019). Namely, striatal DA innervation is involved in the locomotor activity as a part of the extrapyramidal system but also in social interactions as a part of the reward system (Deserno et al., 2015, Báez-Mendoza and Schultz, 2013; Deserno et al., 2015; Felger and Treadway, 2017; Abg Abd Wahab et al., 2019; Lee and Muzio, 2020). The striatum is also the main source of the brain's endogenous opioid neurotransmitter met-enkephalin (Sar et al., 1978; Weisinger, 1995) which in the CNS is involved in limbic system modulation, memory, neuroprotection, centrally mediated analgesia, and stress (Cullen and Cascella, 2022). The dopaminergic signal from VTA acting on the striatum, encourages social interactions (Kopec et al., 2019) hence, attenuated in our experiment dopaminergic synaptic signaling could lead to a decreased propensity for social interactions. Furthermore, Dekeyne et al. (2000) showed that increased serotoninergic neurotransmission can reduce levels of social interaction. These data may explain why in our previous experiment we probably observed withdrawal-like behavior in the vagotomized group with ongoing inflammation. Also, increased striatal met-enkephalin may be involved in social aspects of sickness behavior. Since it is known that striatal met-enkephalin can directly affect

DA release to the limbic system from VTA (Kalivas et al., 1993), increased met-enkephalin in the striatum of our vagotomized group with ongoing peripheral inflammation suggests that this group may be more "neural sensitive" to social stimuli (Moieni and Eisenberger, 2018). Future research should investigate whether sick vagotomized individuals show social self-isolation because it is known that lowered heart rate variability (HRV), a marker of tonic vagal activity, is observed in diseases linked with social withdrawal-like autism spectrum disorder (Cheng et al., 2020) and major depression (Koch et al., 2019).



A proposed explanation for observed changes in striatal neurotransmission. (A) When the vagus nerve is damaged or inefficient, the mechanisms regulating DA release in the striatum do not work properly. This leads to an observed increase in the intracellular metabolism of DA. Under conditions of long-term deficiency of the dopaminergic signal in the striatum, direct serotoninergic projection becomes strengthened and the synthesis of met-enkephalin may increase. (B) Our result shows significant differences in monoaminergic neurotransmission in the right striatum and met-enkephalin in the left one, however, similar tendencies are visible in both hemispheres. Striatum lateralization involves among others differences in dopamine receptor density and affinity. In the left striatum excitatory dopaminergic signal is replaced by an inhibitory serotoninergic signal, resulting in remarkably increased met-enkephalin concentration. In the right striatum, monoaminergic changes are more pronounced however, one inhibitory signal is substituted by another one and met-enkephalin concentration is hardly affected.

Vagotomy disturbs peripheral analgesic mechanism during inflammation

Vagotomy may also influence peripheral inflammation, which in turn may affect sickness behavior. At the early stages of the peripheral inflammatory response, endogenous opioids, mainly β-endorphin and met-enkephalin, are released from activated immune cells (Cabot et al., 2001; Chadzinska et al., 2005; Sehgal et al., 2011). Locally synthesized met-enkephalin modulates immune cell activity (Liang et al., 2016) but also peripheral nociceptive signals reaching the CNS (Corder et al., 2018). β-endorphin and met-enkephalin induce analgesia *via* opioid receptors on peripheral sensory neural fibers and counteracts the hyperalgesic effect of inflammatory mediators such as IL-1β or TNF-α (Cabot et al., 2001; Stein and Land, 2009; Jiang et al., 2015). In a behavioral context, analgesia, especially at the beginning of the inflammatory response, may give animals additional time for searching a safe environment to hide and recover. In fact, in the plasma of the sham group, we observed a significant increase in met-enkephalin concentration in response to LPS injection which may blunt the pain feeling. Our results show that in vagotomized animals, plasma met-enkephalin levels do not increase in response to LPS injection and such an effect may be explained by the influence of vagotomy on different kinds of leukocytes (Table 1).

We think that the lack of enkephalin-mediated analgesia at the early stages of inflammation may contribute to the worsened mood of a sick individual. Such a conclusion agrees with the previously mentioned increased met-enkephalin level in the striatum of vagotomized group with ongoing inflammation, as Gear and Levine (2011) demonstrated that increased met-enkephalin content in NA, in contrast to the blood-borne met-enkephalin, results in hyperalgesia.

CONCLUSIONS

Our results suggest that 30 days after the vagotomy procedure, endogenous opioid analgesic processes at the early stages of the inflammatory response are disturbed. This may affect animal mood, which in turn may have a behavioral implication. Increased striatal serotoninergic and met-enkephalinergic signaling as well as decreased dopaminergic signaling, also suggest that motivation for social interaction might be attenuated. Thus, in conditions of lowered vagal activity, negative subjective perception of inflammatory processes by an animal may be intensified resulting in unnecessary social withdrawal.

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REFERENCES

Abdel-Salam OM, Abdel-Rahman RF, Sleem AA, Mosry FA, Sharaf HA (2013) Effects of afferent and efferent denervation of vagal nerve on endotoxin-induced oxidative stress in rats. J Neural Transm 120: 1673–1688.

Abe C, Inoue T (2018) Role of C1 neurons in anti-inflammatory reflex: Mediation between afferents and efferents. Neurosci Res 136: 6–12.

Abg Abd Wahab DY, Gau CH, Zakaria R, Muthu Karuppan MK, A-Rahbi BS, Abdullah Z, Alrafiah A, Abdullah JM, Muthuraju S (2019) Review on cross talk between neurotransmitters and neuroinflammation in striatum and cerebellum in the mediation of motor behaviour. BioMed Res Int 1767203.

Báez-Mendoza R, Harris CJ, Schultz W (2013) Activity of striatal neurons reflects social action and own reward. Proc Natl Acad Sci 110: 16634–16639.

Báez-Mendoza R, Schultz W (2013) The role of the striatum in social behavior. Front Neurosci 7: 233.

Ben-Shaanan TL, Azulay-Debby H, Dubovik T, Starosvetsky E, Korin B, Schiller M, Green NL, Admon Y, Hakim F, Shen-Orr SS, Rolls A (2016) Activation of the reward system boosts innate and adaptive immunity. Nat Med 22: 940–944.

Berger TW, Kaul S, Stricker EM, Zigmond MJ (1985) Hyperinnervation of the striatum by dorsal raphe afferents after dopamine-depleting brain lesions in neonatal rats. Brain Res 336: 354–358.

Cabot PJ, Carter L, Schäfer M, Stein C (2001) Methionine-enkephalin-and Dynorphin A-release from immune cells and control of inflammatory pain. Pain 93: 207–212.

Capper-Loup C, Rebell D, Kaelin-Lang A (2009) Hemispheric lateralization of the corticostriatal glutamatergic system in the rat. J Neural Transm 116: 1053–1057.

Chadzinska M, Scislowska-Czarnecka A, Pierzchala-Koziec K, Plytycz B (2005) Met-enkephalin involvement in morphine-modulated peritonitis in swiss mice. Mediators Inflamm 2005: 112–117.

Cheng YC, Huang YC, Huang WL (2020) Heart rate variability in individuals with autism spectrum disorders: A meta-analysis. Neurosci Biobeh Rev 118: 463–471.

Cho HU, Kim S, Sim J, Yang S, An H, Nam MH, Jang DP, Lee CJ (2021) Redefining differential roles of MAO-A in dopamine degradation and MAO-B in tonic GABA synthesis. Exp Mol Med 53: 1148–1158.

Corder G, Castro DC, Bruchas MR, Scherrer G (2018) Endogenous and exogenous opioids in pain. Ann Rev Neurosci 41: 453–473.

Cullen JM, Cascella M (2022) Physiology, Enkephalin. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557764/.

Dantzer R (2001) Cytokine-induced sickness behavior: mechanisms and implications. Ann NY Acad Sci 933: 222–234.

Dekeyne A, Denorme B, Monneyron S, Millan MJ (2000) Citalopram reduces social interaction in rats by activation of serotonin (5-HT)(2C) receptors. Neuropharm 39: 1114–1117.

Deserno L, Huys QJ, Boehme R, Buchert R, Heinze HJ, Grace AA, Dolan RJ, Heinz A, Schlagenhauf F (2015) Ventral striatal dopamine reflects behavioral and neural signatures of model-based control during sequential decision making. Proc Natl Acad Sci 112: 1595–1600.

Draper A, Koch RM, van der Meer JW, Aj Apps M, Pickkers P, Husain M, van der Schaaf ME (2018) Effort but not reward sensitivity is altered by acute sickness induced by experimental endotoxemia in humans. Neuropsychopharmacol 43: 1107–1118.

Dunn AJ (2006) Effects of cytokines and infections on brain neurochemistry. Clin Neurosci Res 6: 52–68.

Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR (2010) Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. Biol Psych 68: 748–754.

Elsworth JD, Roth RH (1997) Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol 144: 4–9.

Engler H, Doenlen R, Riether C, Engler A, Niemi MB, Besedovsky HO, del Rey A, Pacheco-López G, Feldon J, Schedlowski M (2009) Time-dependent alterations of peripheral immune parameters after nigrostriatal dopamine depletion in a rat model of Parkinson's disease. Brain Beh Imm 23: 518–526.

Felger JC, Treadway MT (2017) Inflammation effects on motivation and motor activity: role of dopamine. Neuropsychopharmacol 42: 216–241.

Fleshner M, Goehler LE, Hermann J, Relton JK, Maier SF, Watkins LR (1995) Interleukin-1 beta induced corticosterone elevation and hypothalamic NE depletion is vagally mediated. Brain Res Bull 37: 605–610.

Franco R, Casadó-Anguera V, Muñoz A, Petrovic M, Navarro G, Moreno E, Lanciego JL, Labandeira-García JL, Cortés A, Casadó V (2016) Hints on the lateralization of dopamine binding to D1 receptors in rat striatum. Mol Neurobiol 53: 5436–5445.

Gagnon D, Gregoire L, Di Paolo T, Parent M (2016) Serotonin hyperinnervation of the striatum with high synaptic incidence in parkinsonian monkeys. Brain Struct Funct 221: 3675–3691.

Garrett MC, Soares-da-Silva P (1990) Role of type A and B monoamine oxidase on the formation of 3,4-dihydroxyphenylacetic acid (DOPAC) in tissues from the brain of the rat. Neuropharmacol 29: 875–879.

Gear RW, Levine JD (2011) Nucleus accumbens facilitates nociception. Exp Neurol 229: 502–506.

George SR, Kertesz M (1987) Met-enkephalin concentrations in striatum respond reciprocally to alterations in dopamine neurotransmission. Peptides 8: 487–492.

Ghia JE, Blennerhassett P, Collins SM (2007) Vagus nerve integrity and experimental colitis. Am J Physi Gastrointest Liver Physiol 293: 560–567.

Ghizoni DM, João LM, Moratelli Neto L, da Cunha IC, Orlandi Pereira L, Borges FR, Battisti R, de Oliveira LG, Meneghini L, Lucinda AM, Marino Neto J, Paschoalini MA, Faria MS (2006) The effects of metabolic stress and vagotomy on emotional learning in an animal model of anxiety. Neurobiol Learn Mem 86: 107–116.

Guarneri P, Guarneri R, Zarcone D, Bettinazzi G, Amato L, Piccoli F (1985) Lateral differences in the GABAergic system of the rat striatum. Ital J Neurol Scis, 6: 173–176.

Hart BL (1988) Biological basis of the behavior of sick animals. Neurosci Biobeh Rev 12: 123–137.

Herman JP (2018) Regulation of hypothalamo-pituitary-adrenocortical responses to stressors by the nucleus of the solitary tract/dorsal vagal complex. Cell Mol Neurobiol, 38: 25–35.

Hétier E, Boireau A, Dubédat P, Blanchard JC (1988) Neurotensin effects on evoked release of dopamine in slices from striatum, nucleus accumbens and prefrontal cortex in rat. Naunyn-Schmiedeberg's Arch Pharmacol 337: 13–17.

Inagaki TK, Muscatell KA, Irwin MR, Moieni M, Dutcher JM, Jevtic I, Breen EC, Eisenberger NI (2015) The role of the ventral striatum in inflammatory-induced approach toward support figures. Brain Beh Imm 44: 247–252.

Ishizuka Y, Ishida Y, Kunitake T, Kato K, Hanamori T, Mitsuyama Y, Kannan H (1997) Effects of area postrema lesion and abdominal vagotomy on interleukin-1 beta-induced norepinephrine release in the hypothalamic paraventricular nucleus region in the rat. Neurosci Lett 223: 57–60.

Jiang YL, He XF, Shen YF, Yin XH, Du JY, Liang YI, Fang JQ (2015) Analgesic roles of peripheral intrinsic met-enkephalin and dynorphin A in long-lasting inflammatory pain induced by complete Freund's adjuvant in rats. Exp Ther Med 9: 2344–2348.

Kalivas PW, Churchill L, Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience 57: 1047–1060.

Karstaedt PJ, Kerasidis H, Pincus JH, Meloni R, Graham J, Gale K (1994) Unilateral destruction of dopamine pathways increases ipsilateral striatal serotonin turnover in rats. Exp Neurol 126: 25–30.

Kobrzycka A, Napora P, Pearson BL, Pierzchała-Koziec K, Szewczyk R, Wieczorek M (2019) Peripheral and central compensatory mechanisms for impaired vagus nerve function during peripheral immune activation. J Neuroinflamm 16: 150.

Koch C, Wilhelm M, Salzmann S, Rief W, Euteneuer F (2019) A meta-analysis of heart rate variability in major depression. Psychol Med 49: 1948–1957.

Kohls G, Perino MT, Taylor JM, Madva EN, Cayless SJ, Troiani V, Price E, Faja S, Herrington JD, Schultz RT (2013) The nucleus accumbens is involved in both the pursuit of social reward and the avoidance of social punishment. Neuropsychol 51: 2062–2069.

Kopec AM, Smith CJ, Bilbo SD (2019) Neuro-immune mechanisms regulating social behavior: dopamine as mediator? Trends Neurosci 42: 337–348.

Kowalski C, Giraud P (1993) Dopamine decreases striatal enkephalin turnover and proenkephalin messenger RNA abundance via D2 receptor activation in primary striatal cell cultures. Neuroscience 53: 665–672.

Larisch R, Meyer W, Klimke A, Kehren F, Vosberg H, Müller-Gärtner HW (1998) Left-right asymmetry of striatal dopamine D2 receptors. Nucl Med Commun 19: 781–787.

Lee J, Muzio MR (2020) Neuroanatomy, Extrapyramidal System. [Updated 2022 Nov 9]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK554542/.

Liang X, Liu R, Chen C, Ji F, Li T (2016) Opioid system modulates the immune function: a review. Transl Perioper Pain Med 1: 5.

Loehle C (1995) Social barriers to pathogen transmission in wild animal populations. Ecology 76: 326–335.

Maeda T, Kannari K, Shen H, Arai A, Tomiyama M, Matsunaga M, Suda T (2003) Rapid induction of serotonergic hyperinnervation in the adult rat striatum with extensive dopaminergic denervation. Neurosci Lett 343: 17–20.

Maeda T, Nagata K, Yoshida Y, Kannari K (2005) Serotonergic hyperinnervation into the dopaminergic denervated striatum compensates for dopamine conversion from exogenously administered l-DOPA. Brain Res 1046: 230–233.

Manier M, Abrous DN, Feuerstein C, Le Moal M, Herman JP (1991) Increase of striatal methionin enkephalin content following lesion of the nigrostriatal dopaminergic pathway in adult rats and reversal following the implantation of embryonic dopaminergic neurons: a quantitative immunohistochemical analysis. Neuroscience 42: 427–439.

McCusker RH, Kelley KW (2013) Immune-neural connections: how the immune system's response to infectious agents influences behavior. J Exp Biol 216: 84–98.

McQuade R, Sharp T (1997) Functional mapping of dorsal and median raphe 5-hydroxytryptamine pathways in forebrain of the rat using microdialysis. J Neurochem 69: 791–796.

Meiser J, Weindl D, Hiller K (2013) Complexity of dopamine metabolism. Cell Commun Signal 11: 34.

Meitzen J, Pflepsen KR, Stern CM, Meisel RL, Mermelstein PG (2011) Measurements of neuron soma size and density in rat dorsal striatum, nucleus accumbens core and nucleus accumbens shell: differences between striatal region and brain hemisphere, but not sex. Neurosci Lett 487: 177–181.

Mihaylova S, Schweighöfer H, Hackstein H, Rosengarten B (2014) Effects of anti-inflammatory vagus nerve stimulation in endotoxemic rats on blood and spleen lymphocyte subsets. Inflamm Res 63: 683–690.

Mijnster MJ, Raimundo AG, Koskuba K, Klop H, Docter GJ, Groenewegen HJ, Voorn P (1997) Regional and cellular distribution of serotonin 5-hydroxytryptamine2a receptor mRNA in the nucleus accumbens, olfactory tubercle, and caudate putamen of the rat. J Comp Neurol 389: 1–11.

Mitsui T, Fukatsu K, Yanagawa M, Amenomori S, Ogawa E, Fukuda T, Murakoshi S, Moriya T, Yasuhara H, Seto Y (2014) Truncal vagotomy temporarily decreases the pro- and anti-inflammatory cytokine levels in the small intestine. Surg Today 44: 1123–1127.

Moieni M, Eisenberger NI (2018) Effects of inflammation on social processes and implications for health. Ann NY Acad Sci 1428: 5–13.

Muscatell KA, Moieni M, Inagaki TK, Dutcher JM, Jevtic I, Breen EC, Irwin MR, Eisenberger NI (2016) Exposure to an inflammatory challenge enhances neural sensitivity to negative and positive social feedback. Brain Beh Imm 57: 21–29.

Nolan SO, Zachry JE, Johnson AR, Brady LJ, Siciliano CA, Calipari ES (2020) Direct dopamine terminal regulation by local striatal microcircuitry. J Neurochem 155: 475–493.

Okuma Y, Fukuda Y, Osumi Y (1983) Neurotensin potentiates the potassium-induced release of endogenous dopamine from rat striatal slices. Eur J Pharmacol 93: 27–33.

Padovan-Neto FE, Patterson S, F Voelkner NM, Altwal F, Beverley JA, West AR, Steiner H (2020) Selective regulation of 5-HT1B serotonin receptor expression in the striatum by dopamine depletion and repeated L-DOPA treatment: Relationship to L-DOPA-induced dyskinesias. Mol Neurobiol 57: 736–751.

Paxinos G, Watson CH (1998) The rat brain in stereotaxic coordinates. Fourth edition. Academic Press.

Pierzchala K, Van Loon GR (1990) Plasma native and peptidase-derivable Met-enkephalin responses to restraint stress in rats. Adaptation to repeated restraint. J Clin Inv 85: 861–873.

Quirion R, Rowe WB, Lapchak PA, Araujo DM, Beaudet A (1992) Distribution of neurotensin receptors in mammalian brain. What it is telling us about its interactions with other neurotransmitter systems. Ann NY Acad Sci 668: 109–119.

Reardon C, Murray K, Lomax AE (2018) Neuroimmune communication in health and disease. Physiol Rev 98: 2287–2316.

Rivera-Aguilar V, Querejeta E, Jarillo-Luna RA, Reyna-Garfias H, Ponce-Franco D, Milliar-Garcia A, Quiñones-Cárdenas AR, Pacheco-Yepez J, Campos-Rodríguez R (2008) Role of the striatum in the humoral immune response to thymus-independent and thymus-dependent antigens in rats. Immunol Lett 120: 20–28.

Sar M, Stumpf WE, Miller RJ, Chang KJ, Cuatrecasas P (1978) Immunohistochemical localization of enkephalin in rat brain and spinal cord. J Comp Neurol 182: 17–37.

Schiller M, Ben-Shaanan TL. Rolls A (2021) Neuronal regulation of immunity: why, how and where? Nat Rev Immunol 21: 20–36.

Schneider LH, Murphy RB, Coons EE (1982) Lateralization of striatal dopamine (D2) receptors in normal rats. Neurosci Lett 33: 281–284.

Sehgal N, Smith HS, Manchikanti L (2011) Peripherally acting opioids and clinical implications for pain control. Pain Phys 14: 249–258.

Soares-da-Silva P, Garrett MC (1990) A kinetic study of the rate of formation of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the brain of the rat: implications for the origin of DOPAC. Neuropharmacol 29: 869–874.

Stein C, Lang LJ (2009) Peripheral mechanisms of opioid analgesia. Curr Opin Pharmacol 9: 3–8.

Stemick J, Gauer C, Wihan J, Moceri S, Xiang W, von Hörsten S, Kohl Z, Winkler J (2020) Compensatory neuritogenesis of serotonergic afferents within the striatum of a transgenic rat model of Parkinson's disease. Brain Res 1748: 147119.

Stotz EH, Triarhou LC, Ghetti B, Simon JR (1993) Serotonin content is elevated in the dopamine deficient striatum of the weaver mutant mouse. Brain Res 606: 267–272.

Tanaka S, Hammond B, Rosin DL, Okusa MD (2019) Neuroimmunomodulation of tissue injury and disease: an expanding view of the inflammatory reflex pathway. Bioelectron Med 5: 13.

Tanganelli S, Fuxe K, Antonelli T, O'Connor WT, Ferraro L (2001) Cholecystokinin/dopamine/GABA interactions in the nucleus accumbens: biochemical and functional correlates. Peptides 22: 1229–1234.

Thiel CM, Schwarting RK (2001) Dopaminergic lateralisation in the forebrain: relations to behavioural asymmetries and anxiety in male Wistar rats. Neuropsychobiol 43: 192–199.

Tillinger A, Mravec B (2021) Vagotomy affects lipopolysaccharide-induced changes of urocortin 2 gene expression in the brain and on the periphery. Neurochem Res 46: 159–164.

Van de Kar LD, Lorens SA (1979) Differential serotonergic innervation of individual hypothalamic nuclei and other forebrain regions by the dorsal and median midbrain raphe nuclei. Brain Res 162: 45–54.

Walker PD, Capodilupo JG, Wolf WA, Carlock LR (1996) Preprotachykinin and preproenkephalin mRNA expression within striatal subregions in response to altered serotonin transmission. Brain Res 732: 25–35.

Wan W, Wetmore L, Sorensen CM, Greenberg AH, Nance DM (1994) Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. Brain Res Bull 34: 7–14.

Wang ZJ, Rao ZR, Shi JW (1992) Tyrosine hydroxylase-, neurotensin-, or cholecystokinin-containing neurons in the nucleus tractus solitarii send projection fibers to the nucleus accumbens in the rat. Brain Res 578: 347–350.

Waselus M, Galvez JP, Valentino RJ, Van Bockstaele EJ (2006) Differential projections of dorsal raphe nucleus neurons to the lateral septum and striatum. J Chem Neuroanat 31: 233–242.

Weisinger G (1995) The transcriptional regulation of the preproenkephalin gene. Biochem J 307: 617–629.

Widerlöv E, Kilts CD, Mailman RB, Nemeroff CB, Mc Cown TJ, Prange AJ, Breese GR (1982) Increase in dopamine metabolites in rat brain by neurotensin. J Pharmacol Exp Therapeut 223: 1–6.

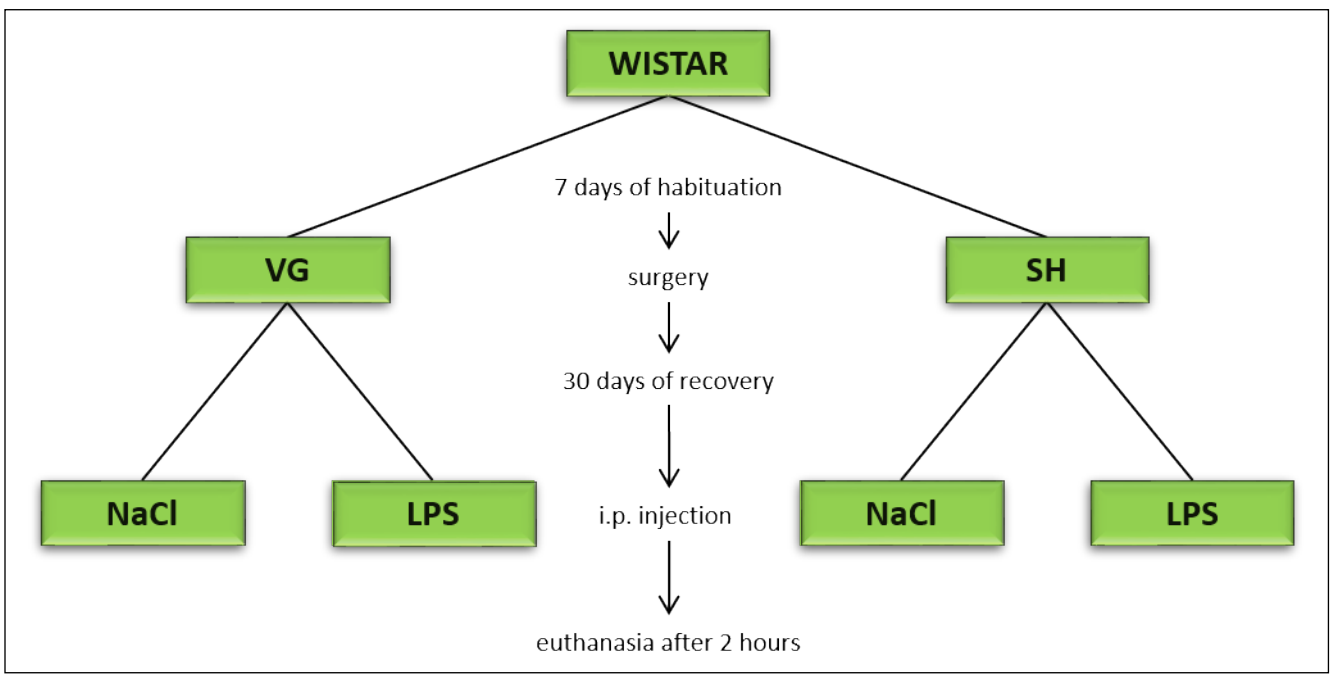
Wieczorek M, Dunn AJ (2006) Effect of subdiaphragmatic vagotomy on the noradrenergic and HPA axis activation induced by intraperitoneal interleukin-1 administration in rats. Brain Res 1101: 73–84.

Yee JR, Prendergast BJ (2010) Sex-specific social regulation of inflammatory responses and sickness behaviors. Brain Beh Imm 24: 942–951.

Yee JR, Prendergast BJ (2012) Endotoxin elicits ambivalent social behaviors. Psychoneuroendocrinol 37: 1101–1105.

Zila I, Mokra D, Kopincova J, Kolomaznik M, Javorka M, Calkovska A (2017) Vagal-immune interactions involved in cholinergic anti-inflammatory pathway. Physiol Res 66: 139–145.

SUPPLEMENTARY MATERIALS



Supplementary Fig. 1. Scheme of the experiment, described in details in Method section. SH – sham surgery, VG – subdiaphragmatic vagotomy, NaCl – i.p. saline injection, LPS – i.p. lipopolysaccharide injection.