Antiseptic Dorogov's Stimulator skin application effect on the animal's behavioral response

Antiseptic Dorogov's Stimulator 3 fraction (ASD) is a thermal processing of animal-origin tissues drug that is used for psoriasis, dermatitis and eczema treatment.

Aim

Evaluation of the antiseptic Dorogov's stimulator 3rd fraction (ASD-3) drug effect on the neuronal activation in the cortex and subcortical zones of the brain of male rats.

Materials and Methods

male Sprague Dawley rats, 2 months, received from vivarium «Pushchino» of Russian Academy of Sciences

storage

vivarium of Centre of Preclinical Research, J.-s.c. «Retinoids» in accordance with GLP principles, 2 weeks quarantine

groups (n=6 in each group)

- 1. drug base
- 2. ASD-3 drug in a dose of 0,5 g/kg/day
- 3. ASD-3 drug in a dose of 4 g/kg/day

drug

Zn paste with 5% ASD-3 for 7 days on shaved interscapular area skin surface

behavioral reactions

Laboras and Sonotrack research instruments (Metris, Netherlands) for motor and USV activity evaluation

anesthesia

«Zoletil 100» (Vibrac, France)

fixation

intracardial immunofix perfusion (BioOptica, Italy) with subsequent fixation in a 15% sucrose solution

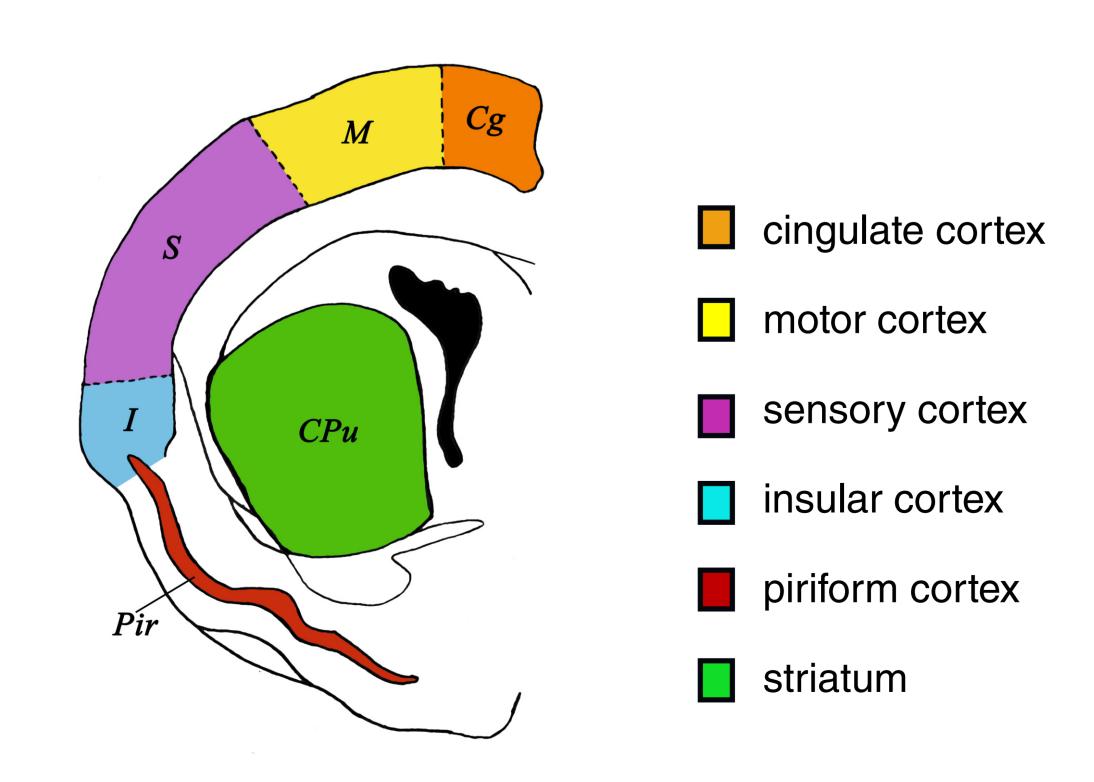


Figure 1. Rat brain regions scheme. Section level – 15

slicing

Thermo Scientific HM430 microtome (Microm GmbH, Germany) with freezing table, thickness – 30 µm

activated neurons evaluation

c-fos protein IHC reaction, indirect avidin-biotin method with 3.3'-diaminobenzidine detection (Santa Cruz Biotechnology Inc., USA)

statistical analysis

mean, its error (M±m) and Student t-criteria, correlation (r) evaluated on Statistica 6.1

structures identification

Paxinos and Watson atlas «The Rat Brain in Stereotaxic Coordinates», 2014, section levels – 13-15

microscopy

Carl Zeiss Axioscope (Carl Zeiss, Germany)

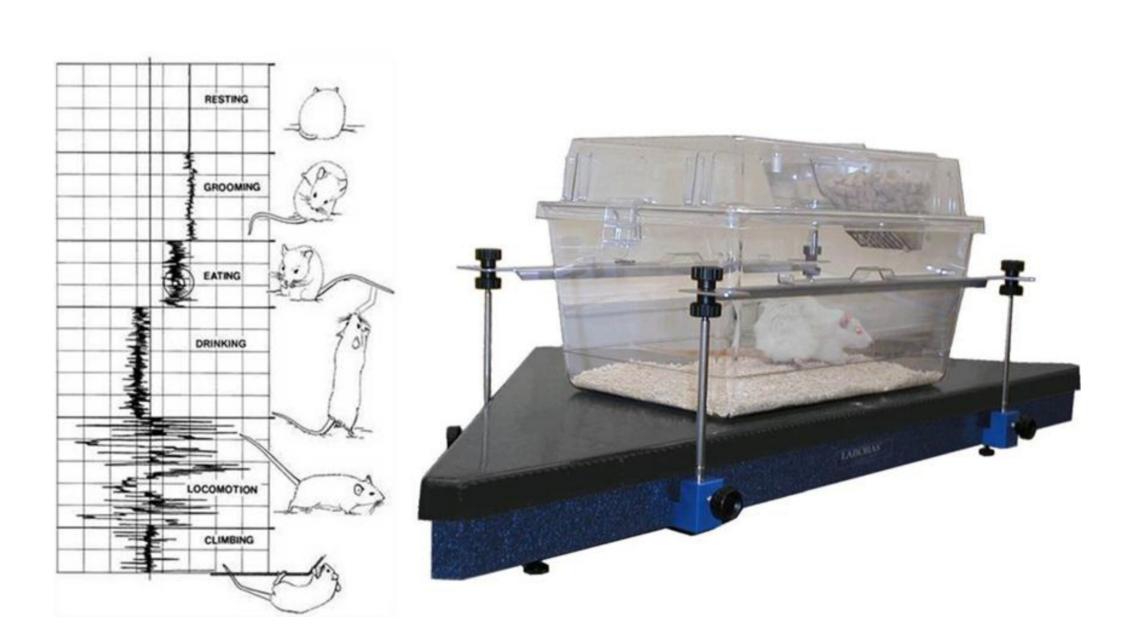


Figure 2. Automated behavior analysis system "Laboras"

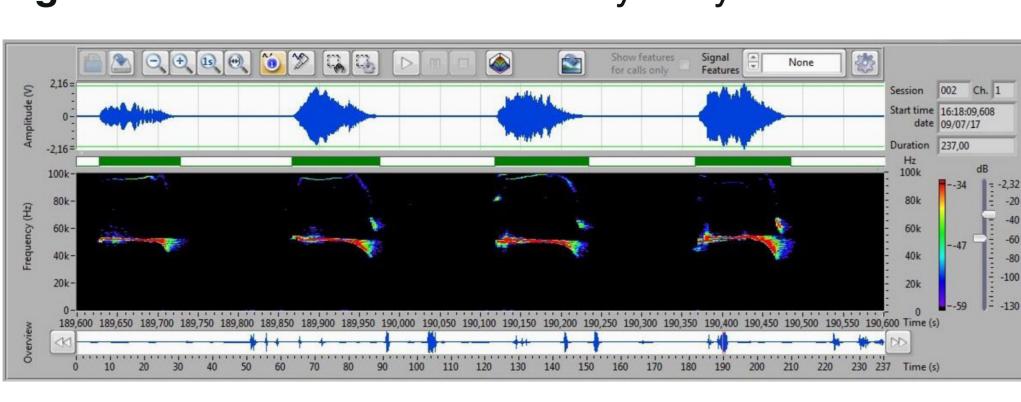


Figure 3. "Sonotrack" USV analysis program window

Results

Some activated cells in the motor cortex associative layer and a significant number of cells in the sensory cortex were observed in the control group. For the drug groups c-Fos positive neurons were found in the sensory cortex (same amount as in control group), and a significant activation of cells was registered in the motor, cingulate and piriform cortex and striatum.

A high number of c-Fos-positive cells were observed in the caudate nucleus head, cingulate cortex and globus pallidus (drug group 1), and in the motor cortex and putamen (drug group 2). Although no local irritating action was mentioned, the average speed, the distance traveled and the number of behavioral acts increased, as well as the ultrasonic vocalizations frequency decreased in according with the control groups in both drug groups.

Reliable correlation between neuronal activation in some brain regions and behavioral response after ASD-3 drug on skin application shows the structural and physiological reactions and proves that there are strong morpho-functional connections.

Duain vaniana	Groups			
Brain regions –	Drug base	ASD-3 drug Group 1	ASD-3 drug Group 2	
cingulate cortex	0.8 ± 0.1	13.4 ± 0.3*	33.3 ± 0.9*#	
motor cortex sensory cortex	2.7 ± 0.2	40.3 ± 1.4*	28.8 ± 0.6*.#	
	4.6 ± 0.2	10.9 ± 0.5*	16.1 ± 0.9*.#	
insular cortex	0	19.0 ± 0.4*	4.2 ± 0.3*#	
piriform cortex	0	19.1 ± 0.7*	8.4 ± 0.5*#	
striatum	0.6 ± 0.1	24.3 ± 0.5*	6.1 ± 0.3*#	

P≤0,05, compared with ASD-3 drug Group 2

Analysis showed the presence of a reliable correlation between number of c-Fos positive neurons and rat's behavioral activity:

` ` ` ` ` ` ` ` ` ` ` ` ` ` ` ` ` ` `	(counted by Laboras system) after 1 week ASD-3 drug on skin application			
Brain regions	Correlation coef. (r)			
motor cortex	0.93			
striatum	0.92			
piriform cortex	0.92			

Correlation between c-Fos positive neurons

in light microscope fields of vision

and average rat speed

Correlation between c-Fos positive neurons in light microscope fields of vision and rat ultrasonic vocalizations (USV) parameters (counted by Sonotrack system) after 1 week ASD-3 drug on skin application

	Brain region	USV parameters	Correlation coef. (r)
	cingulate cortex	Cries (USV)	0.94
		Average frequency, Hz	-0.92
		Peak threshold USV calls	0.91



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ASD-3 drug Group 1

ASD-3 drug Group 2

ASD-3 drug Group 1

ASD-3 drug Group 2

ob. 5x, oc. 20x

Cingulate cortex

Sensory cortex

ob. 40x, oc. 20x





ASD-3 drug Group 1

ASD-3 drug Group 2

ASD-3 drug Group 1

ASD-3 drug Group 2

ASD-3 drug Group 1

ob. 5x, oc. 20x

ob. 5x, oc. 20x

ob. 5x, oc. 20x

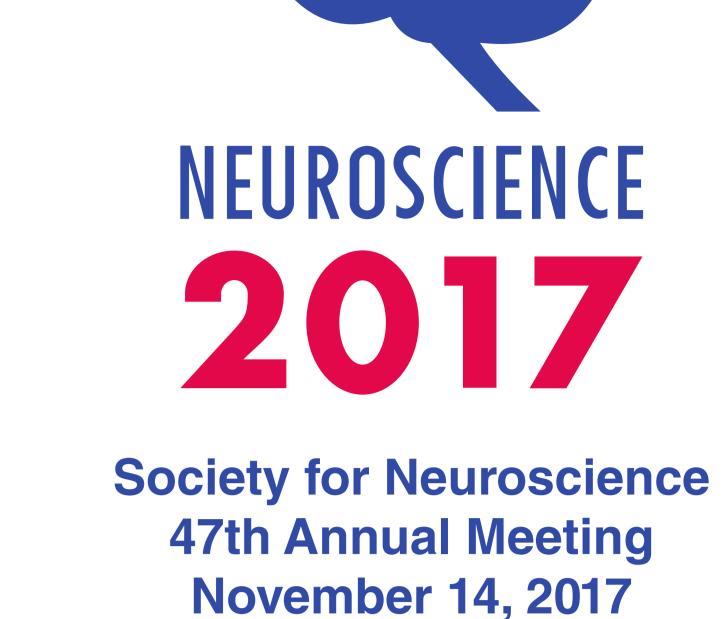
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V. I. NOZDRIN

Motor cortex

Insular cortex



Poster 496.11 / GG26

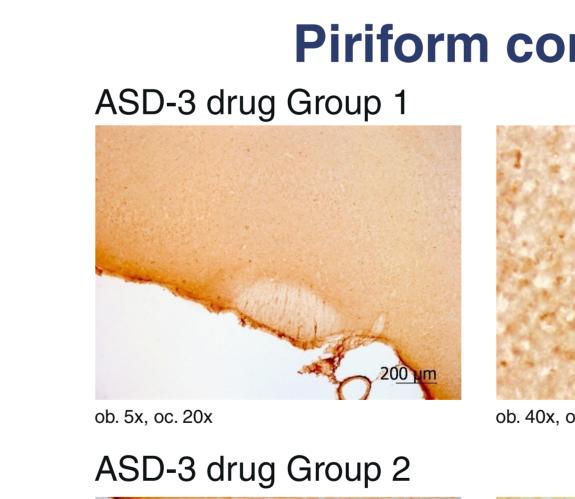


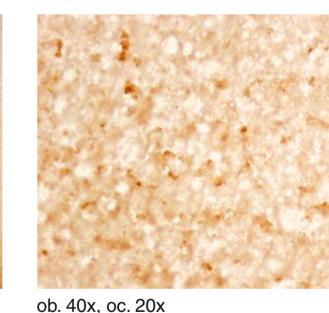
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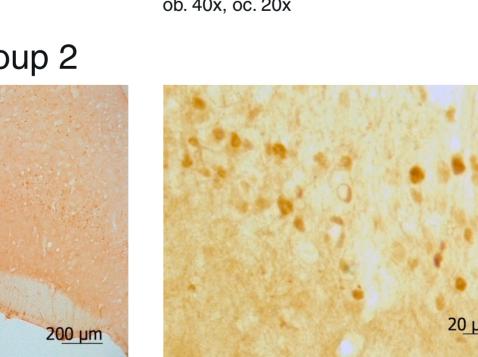




ob. 40x, oc. 20x







ob. 40x, oc. 20x

ob. 5x, oc. 20x ASD-3 drug Group 2

ob. 5x, oc. 20x

Striatum

Conclusion

Finally, it is concluded that ASD application on the skin causes c-Fos expression and an increase of motor activity, which may be interpreted as an indirect drug action on brain structures and behavior.

Activation of the nervous system after the drug skin application indicates the stimulus conduction to the CNS, the transmission pathways of which may be different, including the involvement of peripheral receptors or Langerhans cells.

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Piavchenko, G.A., Dutta, P., Novikova, N.S. et al. Bull Exp Biol Med (2017) 163: 677. © Orel State University © Metris B.V.