ISSN 1580-4003

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

1. Workshop:
UNDERSTANDING CELL COMMUNICATION USING MODERN MICROELECTRONICS

Univerza *v Ljubljani*

1 9 0 1 0 9

Colorado State University

NSF

Slov Vet Res 2010; 47 (4): 129-89

Volume 47 4

Slov Vet Res • Ljubljana • 2010 • Volume 47 • Number 4 • 129-89

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Volume 47 4

Slov Vet Res • Ljubljana • 2010 • Volume 47 • Number 4 • 129-89

The Scientific Journal of the Veterinary Faculty University of Ljubljana

SLOVENIAN VETERINARY RESEARCH
SLOVENSKI VETERINARSKI ZBORNIK

Previously: RESEARCH REPORTS OF THE VETERINARY FACULTY UNIVERSITY OF LJUBLJANA
Prej: ZBORNIK VETERINARSKE FAKULTETE UNIVERZA V LJUBLJANI

4 issues per year / izhaja štirikrat letno

Address: Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia
Naslov: Veterinarska fakulteta, Gerbičeva 60, 1000 Ljubljana, Slovenija
Tel.: +386 (0)1 47 79 100, 47 79 129, Fax: +386 (0)1 28 32 243
E-mail: slovetres@vf.uni-lj.si

Sponsored by the Slovenian Research Agency
Sofinancira: Agencija za raziskovalno dejavnost Republike Slovenije

ISSN 1580-4003
Printed by / tisk: Birografika Bori d.o.o., Ljubljana
Indexed in / indeksirano v: Agris, Biomedicina Slovenica, CAB Abstracts, IVSI
Urlich's International Periodicals Directory, Science Citation Index Expanded,
Journal Citation Reports/Science Edition
http://www.slovetres.si/

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Slov Vet Res 2010; 47 (4)

Slov Vet Res 2010; 47 (3): 133-8
UDC 636.295:577.175.5:159.944

Original Scientific Article

CAN PROLACTIN BE A MEASURABLE MARKER OF STRESS IN DROMEDARIES?

Nalini Kataria*, Anil Kumar Kataria

Department of Veterinary Physiology, Apex Centre for Animal Disease Investigation, Monitoring and Surveillance, College of Veterinary and Animal Sciences, Bikaner- 334 001, Rajasthan, India

*Corresponding author, E-mail: nalinikataria@rediffmail.com

Summary: Prolactin, a hormone produced by the anterior pituitary gland, has a well documented role in milk production and several studies have suggested its role in general adaptation syndrome. As dromedaries *(Camelus dromedarius)* are important animals of arid region, an investigation was carried out in adult dromedaries to assess the role of serum prolactin as a measurable marker of stress. Serum levels of prolactin and cortisol were determined by radioimmunoassay in the healthy and affected dromedaries (those with nasal peg wounds, saddle gall, sand in the third compartment and drought affected). The mean values of serum prolactin (pmol/L) and cortisol (nmol/L) in healthy group were 748.20± 17.82 and 25.93±0.82, respectively. Affected group showed higher levels of serum prolactin and cortisol as compared to healthy group. The mean level of prolactin was 4.94 times higher and cortisol was 4.75 times higher in affected camels as compared to healthy ones. The mean values of different subgroups of affected animals differed significantly and in comparison to healthy male mean value for both serum prolactin ($p \leq 0.01$) and cortisol ($p \leq 0.03$). The camels with sand in their third compartments had highest serum levels of prolactin and cortisol. Increase in serum cortisol suggested that affected camels were stressed and simultaneously many fold rise in serum prolactin clearly suggested that it can be a measurable marker of stress in different affections in dromedaries.

Key words: cortisol; dromedary camel; drought; nasal peg; prolactin; saddle gall; stress

Introduction

Prolactin (PRL) is a single chain polypeptide hormone produced by lactotrophes of anterior pituitary gland and is considered as most versatile pituitary hormone in function that acts directly on different tissues. The physiological actions of prolactin are mediated through specific membrane receptors in the cells of the mammary gland, liver, ovary, testis and prostate (1). Prolactin has been shown to stimulate intestinal calcium absorption, increase bone turnover, and reduce renal calcium excretion (2). Prolactin has multiple metabolic and behavioural effects that may contribute to the general adaptation syndrome as earlier studies (3) have shown the stress induced rise in prolactin secretion in animals and humans. Prolactin induces increased cortisol secretion (4), which is a glucocorticoid secreted by adrenal cortex and is associated with the stress, immune system and thermal regulation (5) besides its important role in many physiological functions including metabolism, mammogenesis, lactogenesis and galactopoiesis (6).

Although every animal has the inherent ability to withstand the stress, the problems arise when the degree of stress exceeds the limit what the body can handle. Generally the response to stress is in the form of neuroendocrine changes involving hormonal and metabolic variations. They can be evoked by anxiety, blood loss, tissue damage, visceral handling, and by the anaesthetic drugs and procedures (7). These reactions can be studied as general adaptation syndrome which enables an animal to adapt itself when suddenly confronted with a critical situation.

The stress syndromes vary in intensity according to the severity of the aggressive stimulus and also

Received: 3 December 2009
Accepted for publication: 31 March 2010

present with different hormone and metabolic profiles, depending on the kind of stressor. Though the camel resists extremes of desert environs, the production potential may became reduced with time (8). Not much attention has been given to either stress factors or health of the camel though stress may alter the physiological status of the individuals (9).For the better management of dromedaries in the stress free environment, different tests which can be carried out to assess the degree of the stress should be established. Acute prolactin responses are related to psychological stress in human being and much of the studies carried out to find the role of prolactin as a stress marker are performed on rats or humans (10, 11). As large animals also suffer from stress, the aim of the present investigation was to understand if prolactin could be an important tool in exploring the physiopathological consequences of certain disease/ affection patterns and a useful neuroendrocine correlate of the individual response to what we define as stress and to assess the role of prolactin in dromedaries as a measurable marker of stress.

Material and methods

The blood samples were collected from 32 adult dromedaries of arid region managed in similar conditions of feeding and watering by the private farmers kept for the purpose of farming and light load carrying. The camels were divided into two groups of 16 each i.e. healthy group and affected group. In the healthy group, the blood was drawn from healthy adult dromedaries of either sex (8 each). The affected group comprised of four sub-groups having males only. The first sub-group constituted of six adult dromedaries having nasal peg wounds, second subgroup of three adults having saddle gall, third subgroup of four drought affected adults and the fourth sub-group of three adults under observations having the history of pica, anorexia and depression, which upon post-mortem revealed the presence of sand in their third compartments.

Sera were separated and analysed for prolactin and cortisol. The serum prolactin was determined by immunoradiometric assay using RIA kit (IRMA CT, RADIM, Italy) following manufacturer protocol. The method uses of two anti-PRL monoclonal antibodies which recognised two different epitopes of the molecule. One antibody was adsorbed in solid phase in the coated tube (mouse monoclonal anti-PRL antibody) and the other as radioactive conjugate labelled with iodine-125 (^{125}I anti-PRL mouse monoclonal antibody in serum matrix). The serum samples and labelled antibodies were incubated simultaneously in the coated tubes. The amount of bound conjugate was directly proportional to the hormone concentration in the sample and standard. At the end of incubation the unbound material was removed by an aspiration and washing cycle (Tris-HCl and Tween 20). The radioactivity in the tubes was measured in a ^{125}I Gamma counter (ECIL,India).

The serum cortisol was determined by using the Gamma coat (^{125}I) cortisol radioimmunoassay kit procedure based on the competitive binding principles of radioimmunoassay (DiaSorin, USA). Serum samples and standards were incubated with cortisol tracer in antibody-coated tubes (Rabbit anti-cortisol serum coated) where the antibody was immobilised onto the lower inner wall of the Gamma Coat Tube. After incubation the contents of the tubes were decanted and the tube was counted in a ^{125}I Gamma counter (ECIL, India).

Statistical significance was assessed between male and female animals of healthy group by paired 't' test (12). As affected animals were comprised of male animals only, their mean values were compared with respective healthy male mean value only. This was carried out by analysis of variance. Further post-hoc (Bonferroni's) test was applied. Mixed model least square and maximum likelihood computer programme PC-I (Copyright, 1987, Walter R. Harvey) were used to determine analyses of variance. Adjustment to multiple comparison was made by Bonferroni's procedure (13,14).

Results

The mean ± SEM values of serum prolactin and cortisol in the dromedaries are presented in table 1.

Serum prolactin value was significantly ($p \leq 0.05$) higher in healthy female camels than in males whereas non significant ($p > 0.05$) change was observed for serum cortisol. Affected groups showed higher levels of serum prolactin and cortisol in comparison to healthy group. The mean rise in prolactin and cortisol levels in affected camels was calculated from that of respective healthy mean value in terms of times. It was 4.94 times higher for serum prolactin level and 4.75 times higher for cortisol. In order to assess the increase in serum prolactin and cortisol in affected animals statistically, analysis of variance was performed which revealed significant changes at 0.01 level of probability for both hormones. Further Bonferroni's adjustments were carried out and the

adjusted probability level was 0.01 for prolactin and 0.03 for cortisol. On this basis it was observed that the mean values of different subgroups of affected camels i.e. nasal peg wounds, saddle gall, drought affected and having sand in the third compartment differed significantly from each other for serum prolactin (p≤0.01) and cortisol (p≤0.03). Each value of affected subgroup differed significantly from respective healthy male mean value of prolactin (p≤0.01) and cortisol (p≤0.03). The camels with sand in their third compartments were having highest serum levels for prolactin and cortisol.

Table 1: Serum levels of prolactin and cortisol in dromedaries. Figures in the parentheses indicate number of animals ('b' = Significant (p≤0.05) variation from healthy male mean value; 'a' = Non significant (p≤0.05) variation from healthy male mean value; 'c' = Significant (p≤0.01) variation from each other for prolactin; 'd' = Significant (p≤0.03) variation from each other for cortisol)

Groups	Sub-groups	Prolactin (pmol/L)	Cortisol (nmol/L)
I. Healthy (16)	Overall healthy mean value	748.20± 17.82	25.93± 0.82
	Male (8)	607.60 ± 21.73[c]	27.03± 1.13[d]
	Female (8)	888.80 ± 26.08[b]	24.83± 1.07[a]
II. Affected (16)	Overall affected mean value (Male)	3004.12±167.0[c]	128.5±7.5[d]
	Nasal peg wounds (6)	1856.8 ±158.40[c]	84.14 ± 8.74[d]
	Saddle gall (3)	2574.3 ±167.2[c]	124.43 ± 6.89[d]
	Drought affected (4)	3220.22± 130.5[c]	140.80± 6.0[d]
	Camels with sand in third compartment (3)	4365.19±217.39[c]	166.09 ± 7.17[d]

Discussion

There is little data in the literature about serum prolactin levels in dromedaries. Commercially available human radioimmunoassay (RIA) kits were used in few studies (15) for PRL determination in one-humped camel (*Camelus dromedarius*) who suggested that serum concentrations of prolactin reflected age and seasonal differences. In the present study the mean value of serum prolactin in healthy camels was similar to those reported by earlier workers in cows (5) whereas it was lower than those reported for ewes (16). Increase in prolactin levels in affected dromedaries indicated that animals were stressed as it was accompanied by a rise in serum cortisol, which is a well documented marker of stress in animals (9). In the present study only one time sampling was carried out as earlier studies suggested consistent secretion pattern (16).

The findings of present investigation regarding an increase in levels of prolactin and cortisol in affected dromedaries were in agreement with the earlier reports where both prolactin and cortisol increased significantly in stressed cows (5) and stressed rats (17). Many other studies have reported an increase in prolactin levels in stressed rats (18). In one study 10-14 fold increase in prolactin secretion was observed after 5 minutes of restraint stress (19). Though cortisol is well established as an important marker of stress (9), in present study prolactin was also found as an important marker in physiological adjustments of stress. Trauma and other affections probably produced a complex set of hormonal and metabolic changes which were evoked by anxiety, blood loss, tissue damage or visceral handling (7).

Increase in prolactin and cortisol could be considered as measurable markers of coping strategies to stress (10). In the affected camels stress most likely developed due to trauma and nervousness which elevated blood prolactin levels and cortisol levels (4). Resutls of our study therefore suggest that prolactin could serve as a sensitive marker of both physical and psychological stress in camels (20). It is important to understand the physiological significance of stress induced prolactin release. Earlier studies (21) have attributed the prolactin surge to the general increase in the adrenergic activity of the hypothalamus which leads to the secretion of PRL-releasing factors (22) and inhibits the tubero infundibular dopaminergic neurons, which are tonic inhibitors of PRL secretion (23). Dopamine regulates cortisol and prolactin secretion in animals (5) and stress-induced PRL release is a rapid and strong response that can be evoked by a large number of medical and surgical procedures (24).

Rise in prolactin levels in affected animals was probably a mechanism to increse the pain threshold (25) and defensive behaviour (26). In camels with sand in their third compartments increased prolactin possible also acted as a protective factor against acute gastric ulceration (4), while raised serum prolactin in camels with nasal peg wounds and saddle gall might have enhanced inflammatory responses (27) as it has been shown before that prolactin has an immuno modulatory effects (28). In drought affected camels scarcity of feed and low quality feed coupled with environmental stress could have been the cause of increased levels of prolactin(29).

Increase in serum prolactin in affected camels suggested its role in stress adaptation to unfavourable conditions. Increase in serum cortisol confirmed that affected camels were stressed. Present study therefore suggest that increase in prolactin could be directly related to the stressful condition of the dromedaries, hence making its measurement a practical stress marker to determine stress in affected dromedaries.

References

1. Reimers TJ. The pituitary gland. In: Pineda MH, ed. McDonald's veterinary endocrinology and reproduction. Iowa: State University Press, 2003: 17–34.

2. Charoenphandhu N, Krishnamra N. Prolactin is an important regulator of intestinal calcium transport. Can J Physiol Pharmacol 2007; 85: 569–81.

3. Fava M, Guaraldi GP. Prolactin and stress. Stress Health 1987; 3: 211–6.

4. Drago F, Amir S, Continella G, Alloro MC, Scapagnini U. Effects of endogenous hyperprolactinemia on adaptive responses to stress. In: MacLeod RM, Thorner MO, Scapagnini, eds. Prolactin, basic and clinical correlates. Padova, Italy: Liviana Press, 1985: 609–14.

5. Ahmadzadeh A, Barnes MA, Gwazdauskas FC, Akers RM. Dopamine antagonist alters serum cortisol and prolactin secretion in lactating Holstein cows. J Dairy Sci 2006; 89: 2051–5.

6. Akers RM. Lactation physiology: a ruminant animal perspective. Protoplasma 1990; 159: 96–111.

7. Reis FM, Ribeiro-de-Oliveira Jr A, Machado LJC, Guerra RM, Reis AM, Coimbra CC. Plasma prolactin and glucose alterations induced by surgical stress: a single or dual response? Exp Physiol 1998; 83: 1–10.

8. Kataria N, Kataria AK. Use of blood analytes in assessment of stress due to drought in camel. J Camel Pract Res 2004; 11: 129–33.

9. Kataria N, Kataria AK, Agarwal VK, Garg SL, Sahani MS, Singh R . Effect of water restriction on serum aldosterone and cortisol in dromedary camel during winter and summer. J Camel Pract Res 2000; 7: 1–7.

10. Sobrinho LG. Prolactin, psychological stress and environment in humans: adaptation and maladaptation. Pituitary 2003; 6: 35–9

11. Caligaris L, Taleisnik S. Prolactin release induced by stress and the influence of oestrogen and progesterone treatments, sex and daily rhythm. Acta Endocrinol 1983; 102: 505–10.

12. Snedecor GW, Cochran WG. Statistical methods. 6th ed. New Delhi: Oxford & IBH Publishing Co, 1967: 45–83.

13. Cobb GW. Introduction to design and analysis of experiments. New York: Springer-Verlag, 1998: 436–81.

14. Sankoh AJ, Huque MF, Dubey SD. Some comments on frequently used multiple endpoint adjustments methods in clinical trials. Stat Med 1997; 16: 2529–42.

15. Al-Qarawi AA, El-Mougy SA. Seasonality and the melatonin signal in relation to age as correlated to the sexual cycle of the one-humped male camel *(Camelus dromedarius).* Biol Rhythm Res 2008 ; 39: 131–42.

16. Notter DR, Chemineau P. Nocturnal melatonin and prolactin plasma concentrations in sheep selected for fertility in autumn lambing. J Anim Sci 2001; 79: 2895–901.

17. Jean Kant G, Mougey EH Pennington LL, Meyerhoff JL. Graded footshock stress elevates pituitary cyclic AMP and plasma β-endorphin, β-LPH, corticosterone and prolactin. Life Sci 2002; 33: 2657–63.

18. Deis RP, Leguizamon E, Jahn GA. Feedback regulation by progesterone of stress-induced prolactin release in rats. J Endocrinol 1989; 120: 37–43.

19. Kjaer A, Knigge U, Warberg J. Histamine and stress induced prolactin secretion: importance of vasopressin V1 and V2 receptors. Eur J Endocrinol 1994; 131: 391–7.

20. Gala R. The physiology and mechanism of stress induced changes in prolactin secretion in the rat. Life Sci 1990; 46: 1407–20.

21. Johnston CA, Spinedi EJ, Negro-Vilar A. Effect of acute etherstress on monoamine metabolism in median eminence and discrete hypothalamic nucleiof the rat brain and on anterior pituitary hormone secretion. Neuroendocrinology 1985; 41: 83–8.

22. Kaji H, Chihara K, Kita T, Kashio Y, Okimura Y, Fujita T. Administration of antisera to vasoactive

intestinal polypeptide and peptide histidine isoleucine attenuates ether induced prolactin secretion in rats. Neuroendocrinology 1985; 41: 529–31.

23. Demarest KT, Moore KE, Riegle GD. Acute restraint stress decrease dopamine synthesis and turnover in the median eminence: a model for the study of the inhibitory neuronal influences on tuberoinfundibular dopaminergic neurons. Neuroendocrinology 1985; 41: 437–44.

24. Noel GL, Suh HK, Stone J, Frantz AG. Human prolactin and growth hormone release during surgery and other conditions of stress. J Clin Endocrinol Metab 1972; 35: 840–51.

25. Ramaswamy S, Pillai, NP, Bapna JS. Analgesic effect of prolactin: possible mechanism of action. Eur J Pharmacol 1983; 96: 171–3.

26. Drago F, Bohus B, Mattheij JAM. Endogenous hypoprolactinemia and avoidance behaviour of the rat. Physiol Behav 1982; 28: 1–4.

27. Matera L, Mori M, Geuna M, Buttiglieri S, Palaestro G. Prolactin in autoimmunity and antitumour defence. J Neuroimmunol 2000; 109: 47–55.

28. Reifen R, Buskila D, Maislos M, Press J, Lerner A. Serum prolactin in celiac disease: a marker for disease activity. Arch Dis Child 1997; 77: 155–7.

29. Brown WB, Forbes JM. Diurnal variations of plasma prolactin in growing sheep under two lighting regimens and the effect of pinealectomy. J Endocrinol 1980; 84: 91–9.

ALI JE LAHKO PROLAKTIN IZMERLJIV OZNAČEVALEC STRESA PRI ENOGRBIH KAMELAH?

N. Kataria, A. K. Kataria

Povzetek: Prolaktin, hormon, ki ga proizvaja adenohipofiza, ima dokumentirano vlogo pri proizvodnji mleka, mnoge študije pa so pokazale še njegovo vlogo pri splošnem sindromu prilagajanja. Ker so enogrbe kamele *(Camelus dromedarius)* pomembne živali na izsušenih področjih, je raziskava potekala na odraslih enogrbih kamelah, z namenom ugotoviti vlogo serumskega proteina kot izmerljivega označevalca stresa. Raven prolaktina in kortizola v serumu so določili z radioimunskim testom pri zdravih in prizadetih enogrbih kamelah (z ranami zaradi nosnih čepov, s sedelnimi odrgninami, s peskom v tretjem predelku želodca ter pri živalih prizadetih zaradi suše). Serumske vrednosti prolaktina (pmol/L) in kortizola (nmol/L) so bile pri zdravi skupini 748.20± 17.82 ter 25.93±0.82. Pri prizadetih kamelah so bile opažene višje ravni serumskega prolaktina in kortizola v primerjavi z zdravo skupino. Srednja vrednost prolaktina je bila 4.94-krat višja ter kortizola 4.75-krat višja pri prizadetih kamelah, v primerjavi z zdravimi. Povprečna vrednost v različnih podskupinah prizadetih živali se je značilno razlikovala od srednje vrednosti pri zdravih živalih za serumski prolaktin ($p \leq 0.01$) in kortizol ($p \leq 0.03$). Kamele s peskom v tretjem predelku želodca so imele najvišje serumske ravni prolaktina in kortizola. Povišan nivo kortizola kaže na to, da so bile prizadete enograbe kamele pod stresom, hkratno povišam nivo serumskega prolaktina pa nakazuje, da je lahko izmerljiv označevalec stresa povzročenega z različnimi dejavniki pri enogrbih kamelah.

Ključne besede: kortizol; enogrba kamela; suša; nosni čep; prolaktin; sedelne odrgnine; stres

Slov Vet Res 2010; 47 (4): 139-44
UDC 611:568.2:537.533.35

Original Scientific Article

LIGHT AND SCANNING ELECTRON MICROSCOPIC STUDY OF THE TONGUE IN THE ZEBRA FINCH *CARDUELIS CARDUELIS* (AVES: PASSERIFORMES: FRINGILLIDAE)

Rahmat Allah Fatahian Dehkordi[1]*, Ali Parchami[1], Shahab Bahadoran[2]

[1]University of Shahrekord, 2-kilometer Saman road, Department of Anatomical Sciences, [2]Department of Poultry Science, Faculty of Veterinary Medicine, Shahrekord, Iran

*Corresponding author, E-mail: fatahian_1349@yahoo.com

Summary: The tongue of adult zebra finch *(Carduelis carduelis)* was examined by light and electron microscopy. The tongue resembles a thick rod with a pointed tip. The length of the tongue is about 8 mm. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue in each bird. A unique feature of the organ is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. The caudal processes are of equal lengths and are tangent to the tongue. Afterwards, the length of the processes increases progressively towards the free point of the organ. The median sulcus is absent on the tongue. Large conical papillae with a W-shaped arrangement are present between the body and the root of the tongue, the apices of which are pointed toward the posterior part of the organ. According to their positions, the PAS-positive compound tubuloalveolar salivary glands can be classified as lingual and laryngeal salivary glands. The lingual salivary glands extend from the lingual apex to the lingual root, whereas the laryngeal salivary glands are situated in both sides of the laryngeal cleft. The ventral side of the tongue is devoid of any glandular structure.

Key words: tongue; papillae; finch

Introduction

The tongue, which plays a very important role in food intake by vertebrates, exhibits significant morphological variations that appear to represent adaptation to the current environmental conditions of each respective habitat (1). In the anatomy of the tongue, three parts may be distinguished: the apex, the body and the root. The body and the root of the organ are demarcated externally by a single or double crest composed of mechanical conical papillae (2, 3). The studies on the structure of the tongue have been conducted on a small number of avian species such as woodpecker (4); cormorants (5); ostrich (6); falcon and kestrel (7); owl (8); white tailed eagle (9); penguin (10) and little tern (11). The results obtained from these studies show a close relationship of the shape of the tongue with the method of food intake and the type of food and habitat.

However, in available literature, there is a lack of morphological data characterizing the structure of the tongue in the zebra finch. The purpose of this study was to describe the morphology of the tongue in this species and to characterize the microscopic structure of the lingual mucosa using light and scanning electron microscopy in order to compare the results with those previous reports in other birds.

Materials and methods

Tongues of 5 adult female zebra finches were used in the investigations. For the observations in the light microscope (LM) the samples of the apex, body and root of the tongue were fixed in the 10% buffered paraformaldehyde (Merck, pH: 7.3) at room temperature for 48 hours and later submitted to the dehydration process in a series of ethanol at increasing concentrations (70-96%) and embedded

Received: 3 September 2010
Accepted for publication: 19 November 2010

in paraplast. Histological serial sections of 7μm of thickness were obtained and stained routinely with haematoxylin-eosin (HE) and periodic acid Schiff (PAS) reaction. The morphometric data were obtained using a KS 400 computer morphometry system (ZEISS). The figures were documented under an Axioscope 2 plus light microscope (ZEISS).

For observations under the scanning electron microscope (SEM) the tongues were rinsed with 0.1M phosphate buffer at pH 7.3. Postfixation was made in 1% osmium tetroxide solution for two hours at 4°C. After dehydration through a graded ethanol series and infiltration by hexamethyl disilazin, the dried specimen were mounted on aluminum stubs and coated using Balzers SCD-040.

The specimens were observed at various angles under a scanning electron microscope (stereoscan 360, Leica Cambridge Ltd., England). The measurement was provided automatically by the SEM unit.

Results

The tongue of the adult zebra finch is about 8 mm long. Overall shape of the tongue resembles a thick rod with a pointed tip. Three parts are distinguished in the dorsal surface of the tongue: the apex, the body and the root of the tongue in each bird. The median sulcus is absent on the tongue of zebra finches (Fig. 1). A unique feature of the organ is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. The caudal processes are of equal lengths and are tangent to the tongue. Afterwards, the length of the processes increases progressively towards the free point of the organ (Figs. 1, 2, 3). At light microscopic level, the muscle bundles of the tongue in the corresponding apical region of the tongue have a V shaped histological arrangement in cross section (Fig. 8).

Large conical papillae are present between the body and the root of the tongue, the apices of which are pointed toward the posterior part of the organ. These mechanical papillae are arranged like the letter W at the edges of two huge caudally directed elevations of the lingual corpus. The axial papillae are noticeably smaller and thinner than the abaxial ones (Figs 1, 6).

The mucosa of the whole dorsal and ventral surface of the apex, body and root of the tongue is covered with flat stratified non-keratinised epithelium (Figs. 8, 9). At electron microscopic level, the mucosal surface of the tongue in the apex, body and root of the tongue is flat with no papillae (Figs. 1, 2, 4, 5, 6, 7). The special delicate pattern of microridges can be seen at electron microscopic level on the surface of the tongue particularly in the body and root regions (Figs. 4, 7). Gustatory papillae are not found in the epithelium covering the tongue in the finch.

The salivary glands are located in the lamina propria beneath the dorsal lingual epithelium. According to their positions, these glands can be classified as lingual and laryngeal salivary glands. The lingual salivary glands extend from the lingual apex to the lingual root, and are interspersed between the stratified squamous lining the dorsal surface and the lingual muscle bundles of the tongue (Figs. 8, 9). Dorsal lingual epithelium overlying the lingual salivary glands is considerably thicker than that the other parts (Fig. 8). Lingual salivary glands are divided into two portions (laryngeal salivary glands) by the laryngeal cleft (Fig. 10). Both lingual and laryngeal salivary glands are of compound tubuloalveolar type consist of secretory endpieces composed of tall columnar cells with flattened nuclei at their basis. The glandular cells rest at a delicate basement membrane, having extensively vesicular cytoplasm, and thus stained lighter with haematoxylin and eosin stain. Secretory units forming the glands are separated by narrow connective tissue septa containing capillary vessels (Fig. 9). The ducts of the lingual glands opened onto the dorsal surface of the tongue. The secretory cells of the both lingual and laryngeal salivary glands reacted positively to PAS reaction (Fig. 9). The ventral side of the tongue is devoid of any glandular structure (Fig. 8).

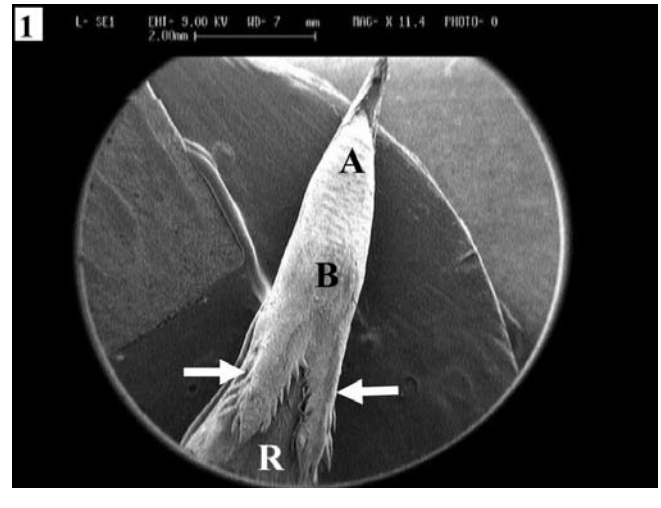

Figure 1: Scanning electron micrograph of the dorsal surface of the zebra finch tongue. Three parts are distinguished in the tongue: lingual apex (A), lingual body (B) and lingual root (R). Note the W-shaped arrangement of the conical papilla (arrows) between the lingual apex and body

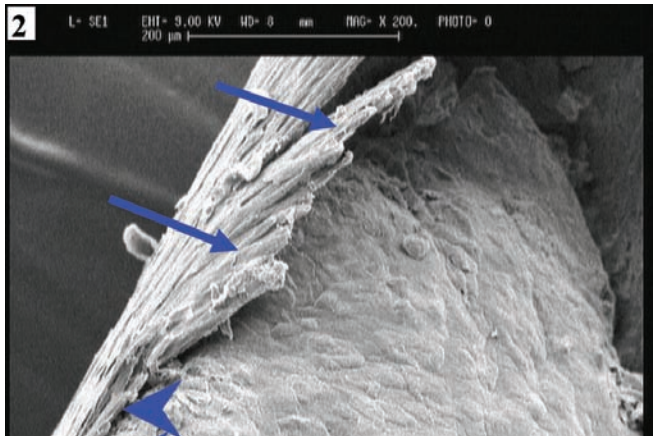

Figure 2: Scanning electron micrograph of the surface of the lingual apex, showing many needle-like processes in the lateral side of lingual apex (arrows). Note that the caudal processes (arrowheads) are completely tangent to the organ. The lingual surface is flat with no lingual papilla

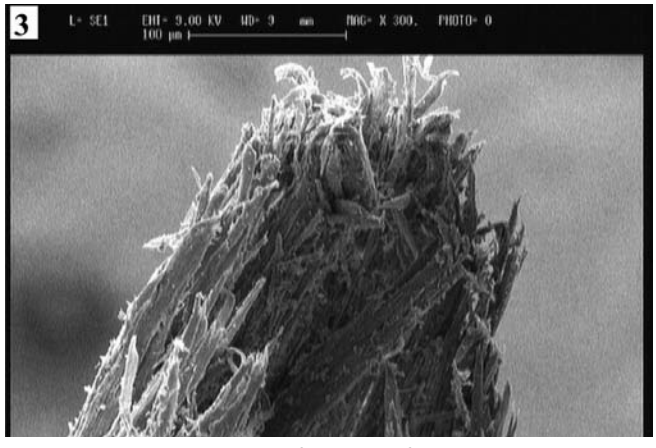

Figure 3: A higher magnification of the needle-like processes of the apex of the tongue

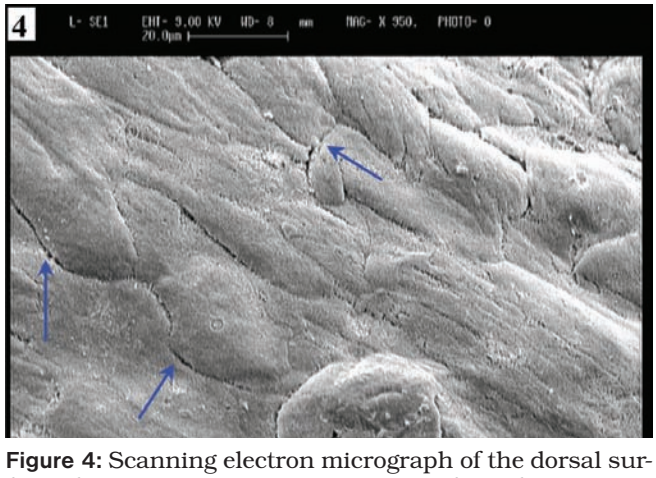

Figure 4: Scanning electron micrograph of the dorsal surface of the lingual body. The dorsal surface of the lingual body presents smooth aspect with no papilla. Note the borders between surface squamous cells (arrows) and microridges on the surface epithelium

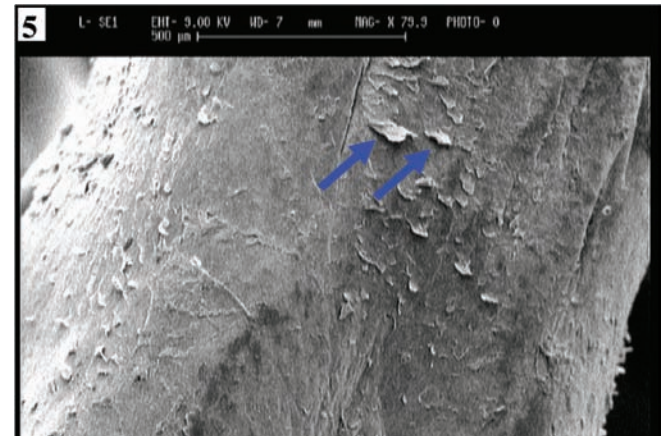

Figure 5: Scanning electron micrograph of the dorsal surface of the body of the tongue. The arrows show the desquamate cells of the non-keratinized epithelium

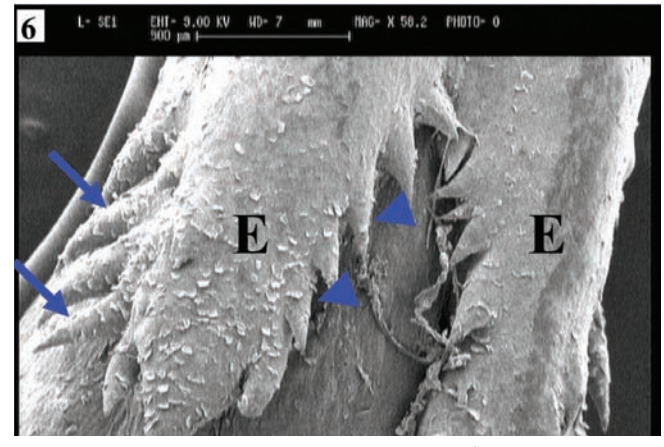

Figure 6: Scanning electron micrograph of the large conical papilla between the body and the root of the dorsal surface of the tongue. Note that the abaxial papillae (arrows) are considerably larger than the axial ones (arrowheads). Desquamated cells are clearly visible on the surface of two huge caudally directed extensions (E) from the lingual body

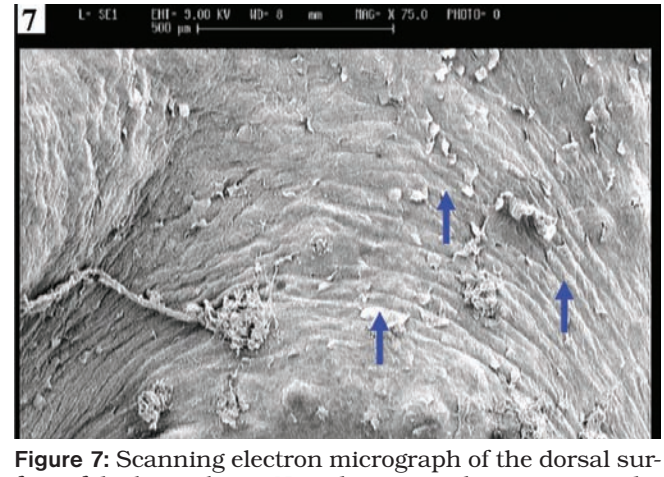

Figure 7: Scanning electron micrograph of the dorsal surface of the lingual root. Note the microridge pattern on the surface epithelium (arrows)

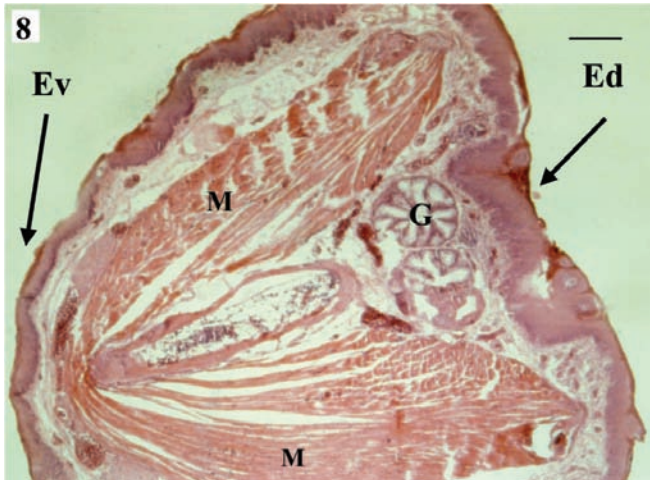

Figure 8: Cross section of the apex of the tongue; light photomicrograph, hematoxylin and eosin staining. Ed, dorsal epithelium; Ev, ventral epithelium; G, lingual salivary glands. Note the v-shaped arrangement of the skeletal muscle bundles (M). Scale bar, 100 μm

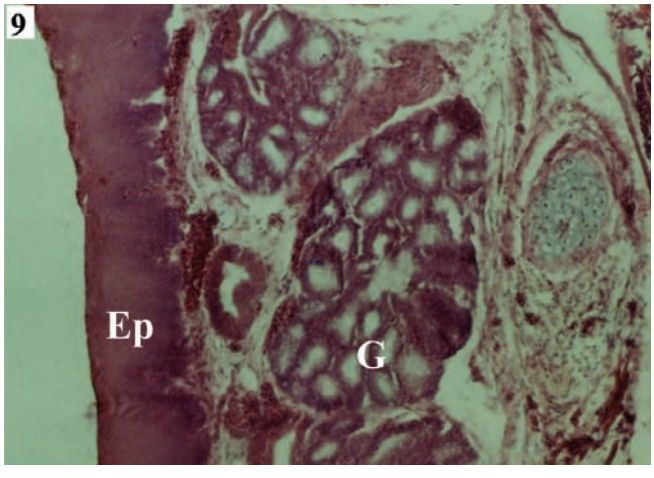

Figure 9: Cross section of the body of the tongue; light photomicrograph, PAS staining. Dark cytoplasm in the cells of the lingual salivary glands (G) presents a positive PAS reaction. Ep, dorsal lingual epithelium. Scale bar, 70 μm

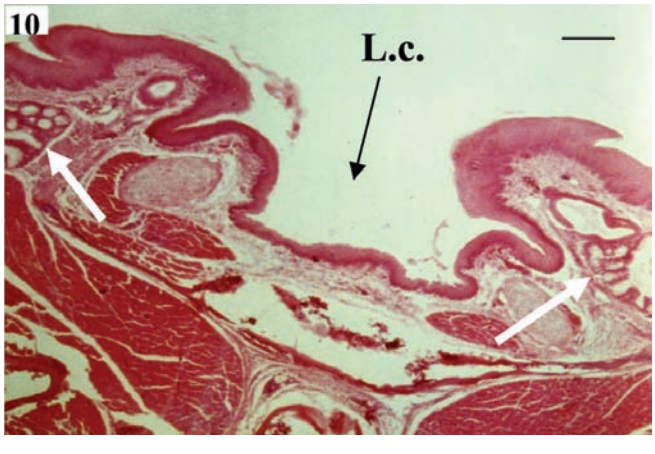

Figure 10: Dorsal surface of the tongue. The laryngeal salivary glands (arrows) at both sides of the glottis-laryngeal cleft (L.c.). hematoxylin and eosin staining. Scale bar, 40 μm

Discussion

General morphological features of the tongue in the zebra finch show considerable structural differences in comparison with the tongues of species of birds investigated previously.

The shape of the tongue in birds is a species specific trait (3, 12). Tongues used to manipulate food, such as in piscivorous species, are nonprotruding and covered with stiff, sharp, caudally directed papillae. In birds of prey, the tongue is a rasp-like structure with the rostral portion frequently being very hard and rough. On the tongue of birds that typically strain food particles (e.g. ducks), the rostral portion forms a scooplike structure with the lateral borders having a double row of overlapping bristles. The bristles work in conjunction with the lamellae of the bill to filter particles (13). In both lateral sides of anterior lingual apex in the Japanese pygmi woodpecker, some conical processes are observed and in the posterior part of the lingual apex, there are many needle processes, the apices of which are pointed towards the posterior part of the tongue (4).

Results obtained from the present study showed that a unique feature of the tongue in zebra finch is the presence of many fine densely populated needle–like processes in both lateral sides of the anterior lingual apex. These processes may help bird in direct food caudally towards the caudal parts of the oropharyngeal cavity. Peculiar V-shaped arrangement of skeletal muscle bundles found in the apex of the tongue show that the bird can move the papillae in appropriate directions. There have been no reports regarding rostrally directed needle processes of the lingual apex in various birds.

The median groove is a characteristic feature found on the tongue of white tailed eagle, ducks and geese, whereas it is absent on the tongue of chickens, pheasants and penguins (2, 3, 9, 14, 15). On the dorsal surface of the short tongue of the Cormorant, in the midline a crest is found, resembling a ridge, reaching both ends of the organ (5). Our results also showed that the median sulcus is absent on the tongue of zebra finches.

In most of the species of birds examined, the tongue except for its apical part, is covered by a flat epithelium. Emura *et al.* (2009) stated that in pygmi woodpecker, the dorsal surface of the lingual body presents smooth aspect (4). In penguins, the whole dorsal lingual surface is covered by long conical papille that help to hold ingested food (10). Many processes were observed densely distributed over

the entire anterior 2/3 of the lingual dorsal surface in the chicken tongue (14), over the entire lingual apex of the dorsal surface, except in the tip of the apex in the owl (8) and over the entire lingual apex in the peregrine falcon and common kestrel (7). The results obtained from the present study also showed that the whole dorsal surface of the tongue of the finch is flat and completely devoid of lingual papillae.

As shown by the light microscopic studies on the tongue in the zebra finch, the mucosa of the whole dorsal and ventral surface of the apex, body and root of the tongue is covered with flat stratified non-keratinised epithelium. This finding is in accordance with that of Jackowiak and Ludwig in study of the ostrich tongue (9). In most of the other species of birds examined, the whole dorsal surface of the tongue up to conical papillae is covered by horny epithelium, whereas the stratified epithelium without the horny layer usually covers a part of the root of the tongue (14-16). Microridges, found on superficial cells over the entirety of the finch's tongue, have been described in both mammals and in birds. Microridges have been interpreted as structures that increase the adhesion of mucus to the epithelium (6).

Our results showed that large conical papillae are found in the posterior part of the lingual body, the apices of which were pointed towards the posterior part of the tongue. It has been reported that development of lingual conical papillae of avian species is related to their feeding habits and the crest of the papillae is well developed in birds such as white tailed eagle and owl which feed on fish or small animals and is absent in birds such as woodpecker and ostrich which feeds on insect or plants (6-9). Zebra Finches are primarily seed-eating birds, as their beaks are adapted for dehusking small seeds. They prefer millet, but will consume many other kinds of seeds as well. The present results show that this species of birds has very well-developed conical papillae despite the fact that it feeds on seeds. The discrepancy between the results might be due to the genetic variations in the different avian species. However more work is needed for explanation. It also needs to be added that there have been no reports regarding conical paillae with peculiar W-shaped arrangement in tongues of species of birds investigated previously.

Salivary glands also show considerable species variation in birds. While salivary glands are generally well developed in granivorous species, they are less developed in birds of prey, poorly developed in piscivores, and absent in the Anhinga and Great Cormorant (13). The results of studies on the distribution of lingual glands, conducted so far on few bird species, make it possible to distinguish anterior and posterior lingual glands (15-17). In the Ostrich, however, the lamina propria of the lingual mucosa is filled with mucous glands whose openings are found on both the dorsal and ventral surface of the tongue (6). The localization of the compound tubuloalveolar lingual salivary glands of zebra finch seems to be a species-specific trait since the glands exist beneath the entire surface of dorsal lingual epithelium and their ducts opened onto the dorsal surface of the tongue. The ventral side of the tongue is devoid of any glandular structure. The secretory cells of the lingual salivary glands show strongly positive reaction to PAS reaction, indicating that the saliva of the finch similar to that of other birds is rich in glycoproteins.

Acknowledgment

This work was financially supported by the University of Shahrekord, Iran.

References

1. Iwasaki S. Evolution of the structure and function of the vertebrate tongue. J Anat 2002; 201(1): 1–13.

2. Komarek V, Malinovesky L, Lemez L. Anatomia avium domesticarum et embryologia galli. Vol. 2/3. Bratyslava: Priroda vedavatel'stvo knih a casopisov, 1982.

3. Vollmerhaus B, Sinowatz F. Verdauungsapparat. In: Nickel R, Schummer E. Seiferle E, eds. Lehrbuch der Anatomie der Haustiere. Bd. 5. Anatomie der Vögel. Berlin: Parey, 1992: 202–12.

4. Emura S, Okumura T, Chen H. Scanning electron microscopic study of the tongue in the Japanese pygmy woodpecker *(Dendrocopos kizuki).* Okajimas Folia Anat Jpn 2009; 86: 31–5.

5. Jackowiak H, Andrzejewski W, Godynicki S. Light and scanning electron microscopic study of the tongue in the cormorant *Phalacrocorax carbo* (Phalacrocoracidae, Aves). Zool Sci 2006; 23: 161–7.

6. Jackowiak H, Ludwig M. Light and scanning electron microscopic study of the structure of the ostrich *(Strutio camelus)* tongue. Zool Sci 2008; 25 (2): 188–94.

7. Emura S, Okumura T, Chen H. Scanning electron microscopic study of the tongue in the per-

egrine falcon and common kestrel. Okajimas Folia Anat Jpn 2008; 85: 11–5.

8. Emura S, Chen H. Scanning electron microscopic study of the tongue in the owl *(Strix uralensis).* Anat Histol Embryol 2008; 37: 475–8.

9. Jackowiak H, Godynicki S. Light and scanning electron microscopic study of the tongue in the white tailed eagle *(Haeliaeetus albicilla,* Accitripidae, Aves). Ann Anat 2005; 187: 197–222.

10. Kobayashi K, Kumakura M, Yoshimura K, Inatomi M, Asami T. Fine structure of the tongue and lingual papillae of the penguin. Arch Histol Cytol 1998; 61(1): 37–46.

11. Iwasaki S. Finestructure of the dorsal lingual epithelium of the little tern, sterna-albifrons pallas (aves, lari). J Morphol 1992; 212: 13–26.

12. Campbell B, Lack E. A dictionary of birds. Calton: The British Ornithologists' Union, T. & A. D. Poyser, 1985.

13. Whittow GC. Sturkie's avian physiology. NewYork, London: Academic Press, 2000.

14. Iwasaki S, Kobayashi K. Scanning and transmission electron microscopy studies on the lingual dorsal epithelium of chickens. Kaibogaku zasshi1 1986; 61(2): 83–96.

15. McLelland J. Aves digestive system. In: Getty R, ed. Sisson and Grossman's the anatomy of the domestic animals. Vol. 2. 5th ed. Philadelphia: Saunders Company, 1975: 1857–82.

16. Homberger DG, Meyers R. Morphology of the lingual apparatus of the domestic chicken *Gallus gallus,* with special attention to the structure of the fasciae. Am J Anat 1989; 186: 217–57.

17. Vollmerhaus B, Sinowatz F. Verdauungsapparat. In: Nickel R, Schummer E. Seiferle E, eds. Lehrbuch der Anatomie der Haustiere. Bd. 5. Anatomie der Vögel. Berlin: Parey, 1992:

PROUČEVANJE JEZIKA LIŠČKA *CARDUELIS CARDUELIS* (AVES: PASSERIFORMES: FRINGILLIDAE) S POMOČJO SVETLOBNE IN VRSTIČNE ELEKTRONSKE MIKROSKOPIJE

R.A.F. Dehkordi, A. Parchami, S. Bahadoran

Izvleček: V članku je bil proučevan jezik odraslega liščka *(Carduelis carduelis)* s pomočjo svetlobne in vrstične elektronske mikroskopije. Jezik je podoben debeli palici z zašiljeno konico. Dolžina jezika je približno 8 mm. Pri vsaki ptici je dorzalna površina jezika ločena na tri dele: konico, telo in koren jezika. Posebna značilnost organa je prisotnost mnogih finih, na gosto razporejenih, kot igla tankih podaljškov na obeh vzdolžnih straneh jezika. Dolžina podaljškov stopnjujoče narašča proti prostemu koncu organa. Na jeziku ni sredinskega žleba. Velike stožčaste okušalne brbončice so v obliki črke W razporejene med telesom in korenom jezika, njihova konica pa je obrnjena proti zadnji strani organa. Glede na njihov položaj lahko PAS-pozitivne sestavljene cevkasto-mešičkaste slinske žleze razvrstimo v jezične in žrelne slinske žleze. Jezične slinske žleze segajo od konice do korena jezika, medtem ko so žrelne slinske žleze na obeh straneh žrelne razpoke. Na ventralni strani jezika ni žlez.

Ključne besede: jezik; brbončice; ščinkavec

Articles and abstracts in this section are published as proceedings of 1. Workshop:

UNDERSTANDING CELL COMMUNICATION USING MODERN MICROELECTRONICS

Ljubljana, Slovenia, August 23. – 24. 2010

Editors: Gregor Majdič, Tom Chen, Stuart A. Tobet

Workshop was supported by National Science Fundation, USA.

This program is based in part upon collaborative work supported by a National Science Foundation Grant No. 0841259; Colorado State University, Thomas Chen, Principal Investigator, Michael A. de Miranda and Stuart Tobet Co-Principal Investigators. Any opinions, findings, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Slov Vet Res 2010; 47 (4): 147
UDC 576:577

A MULTI-DISCIPLINARY RESEARCH AND TEACHING PROGRAM IN BIOMEDICAL ENGINEERING FOR DISCOVERY AND UNDERSTANDING OF CELL COMMUNICATION

Stuart Tobet, Charles Henry, Michael DeMiranda, Thomas Chen

School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA

This project provides an innovative program for developing a new generation of scientists in biomedical science and engineering that are trans-disciplinary in their training, better equipped for multilevel communication across ages (GK-12) and fields (e.g., industry interaction), and finally prepared to take leadership roles for scientific inquiry and progress into the 21st century. The research component consists of activities in sensing, modeling, and understanding how molecules move and the functions of multi-cellular tissues and organ systems in response to external chemical and physical stimuli through intercellular communication. The research project focuses on studying the release of key molecules of intercellular communication in brain, pituitary, and gonads and their effects on cell behavior. Additional project components also examine ways in which such data can be modeled and interpreted for maximum understanding of complex processes. Among the molecules of interest, there are major advantages for biosensor technologies that are amenable to electrochemical detection. (e.g., 1). The project is particularly interested in the detection of molecular gradients in extracellular space that are essential for the development of tissue and organ systems as well as marking the response to external chemical and physical stimuli (2, 3). Such gradients are difficult to detect because molecules released into extracellular space are not readily fixable in space by the vast majority of histological methods (4). Advanced silicon technology is being used to build dense biosensor arrays with the resolution of single cells, and that can operate at high frequencies to achieve sufficient temporal resolution to visualize molecules released to communicate between cells.

The results of the microscopic approach must be interrogated using state of the art techniques of data and image analysis. Using in vitro slice preparations from developing mammalian embryos, there are multiple patterns of cell migration in different brain regions (e.g., 5, 6, 7), including significant cell mixing, and the identification of apparent boundaries. The number of different fluorophores useful for cellular level imaging has exploded over the last 10 years. Visual imaging is being combined with electrochemical methods to yield synergisms in molecular information processing. Micro-sized biosensor arrays will allow the detection of small currents with micron resolution, and yield chemical data to complement optical methods.

The broad impact of the program is three-fold: 1) the research is critical for continued understanding and advances in fundamental questions facing biology and medicine; 2) it provides a broad framework for incorporating biomedical engineering research in K-12 STEM (Science, Technology, Engineering, and Math) curriculum; and 3) it demonstrates the power of broad partnerships between universities, K-12 education districts, local industry, and international collaboration on improving graduate and K-12 education.

Supported by NSF DGE-0841259.

References

1. Liu Y, Vickers JA, Henry CS. Simple and sensitive electrode design for microchip electrophoresis/electrochemistry. Anal Chem 2004; 76: 1513–7.

2. Wolpert L. One hundred years of positional information. Trends Genet 1996; 12(9): 359–64.

3. Gurdon JB, Bourillot PY. Morphogen gradient interpretation. Nature 2001; 413(6858): 797–803.

4. Tobet S, Henderson R, Whiting P, Sieghart W. Special relationship of gamma-aminobutyric acid to the ventromedial nucleus of the hypothalamus during embryonic development. J Comp Neurol 1999; 405: 88–98.

5. Bless E, Walker H, Yu K, et al. Live view of gonadotropin-releasing hormone containing neuron migration. Endocrinology 2005; 146: 463–8. *[cell movement video available from Journal Website].*

6. Knoll JG, Wolfe CA, Tobet SA. Estrogen modulates neuronal movements within the developing preoptic area-anterior hypothalamus. Eur J Neurosci 2007; 26:1091–9.

7. McClellan KM, Calver AR, Tobet SA. GABAB receptors role in cell migration and positioning within the ventromedial nucleus of the hypothalamus. Neuroscience 2008; 151: 1119–31.

Slov Vet Res 2010; 47 (4): 149-54
UDC 621.317

Original Scientific Article

LOW NOISE SIGNAL PROCESSING FOR MEMS/NEMS BASED CHEMICAL/BIOLOGICAL SENSORS: A SYSTEM PERSPECTIVE

Drago Strle

Laboratory for Microelectronics, Faculty for Electrical Engineering, University of Ljubljana, Tržaška 25, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: drago.strle@fe.uni-lj.si

Summary: The paper presents architecture, design, modeling and implementation of low-noise signal processing electronics needed to implement high-resolution MEMS/NEMS based capacitive sensors interfaces, which can be used for chemical/biological sensor interface. Using double-mixing lock-in amplifier principle with signal processing appropriately distributed between analogue and digital circuitry, using low noise charge amplifier, low-noise programmable gain stage, several low-noise filters, 2nd order ΣΔ modulator and the DSP a very high SnR is achieved. With proposed architecture, it is possible to sense a capacitance difference smaller than 0.5aF/√Hz on a 2pF feedback capacitors with SnR (1Hz) better than 134dB. Because of achieved characteristics it is possible to use proposed interface for sensing capacitance changes of micro/nano scale based chemical and/or biological sensors with ultimate sensitivity. The circuitry is implemented in 0.350μm BCD technology and occupies approx. 2.5 mm^2. Using this electronics together with chemically modified MEMS COMB capacitive sensor the measured detection level is better than 5ppb vapor molecules in the air at room temperature.

Key words: electronic bio sensors interface; noise in bio sensor interfaces; capacitive sensor interface; lock-in-amplifier

Introduction

Many MEMS/NEMS sensors utilize capacitive based transduction mechanism to sense different physical, chemical or biological effects (1-3). Usually, the capacitance change is very small, so the signal processing electronics must be such that SnR from the sensor is not reduced considerably. In addition, temperature drift, power consumption and silicon area must all be as small as possible. The most critical blocks are: the sensor, input amplifiers and ADCs, where thermal and 1\f noise, offset, and quantization noise can degrade the performances. The quantization noise is made negligible compared to other noise sources, thus the main noise contributions are coming from the first stages of the analog signal processing blocks. Different techniques exist for the reduction of offset voltage and 1/f noise: AZ, CDS and CHP (4). In AZ and CDS the offset voltage and 1/f noise are attenuated while thermal noise is increased. CHP (chopping) is better compared to other techniques but also in this case the thermal noise increases approx. by factor of 2. The problem is the residual offset if such signal is amplified. In this work we do not use chopping, instead, sensing signals are square-waves connected directly to the sensing capacitances, so the sensor signals (capacitances) are immediately transferred around multiples of f_s, which must be above corner frequency of 1/f noise. To reduce power consumption the analog HF signal processing is used in first stages only; in later stages the signal frequency is reduced by down-mixing to f_o before the ADC. The DSP reduces signal frequency down-to the DC. In this way a special version of lock-in amplifier principle is implemented.

This paper is organized as follows. In section sensor a capacitive COMB sensor used in our experiments is presented together with estimates of required sensitivity. The architecture of proposed signal processing electronics is given section Architecture and noise together with estimated SnR and most important system level simulation results. Section Modelling and measurements deals with

Received: 30 August 2010
Accepted for publication: 17 September 2010

modeling, some additional simulation results and measurements. In Conclusions a possibilities for further improvements are presented.

Sensor

Figure 1 shows SEM micrograph of a differential COMB sensor. The sensor is connected as suggested on Figure 2. The capacitance is $C_0 = 0.5pF$ and production spread is smaller than $\varepsilon_p \leq 5\%$. Capacitor C_p is modified by appropriate molecule that is self-assembled on C_p, while C_n is unchanged. A compatible molecules in the air or in the liquid are adsorbed on the surface of C_p and its capacitance changes to C_{p1}. Assuming that the thickness of one layer of adsorbed molecules is approx. 0.1nm the capacitor change $\delta C_p = C_{p1} - C_{p0}$ can be calculated using equation (1):

$$\delta C_p = C_0 \left(1 + \frac{\varepsilon_p}{2}\right)\left[\frac{\delta}{(d-\delta)}\right] \leq 33aF \qquad (1)$$

The difference of capacitors $\Delta C = C_p - C_n$ before and after adsorption can be calculated by (2) and is carrying the information.

$$\Delta C_1 - \Delta C_0 = C_0 \cdot \varepsilon_p + \left\{C_0 \left(1 + \frac{\varepsilon_p}{2}\right)\frac{\delta}{d-\delta}\phi\right\} \qquad (2)$$

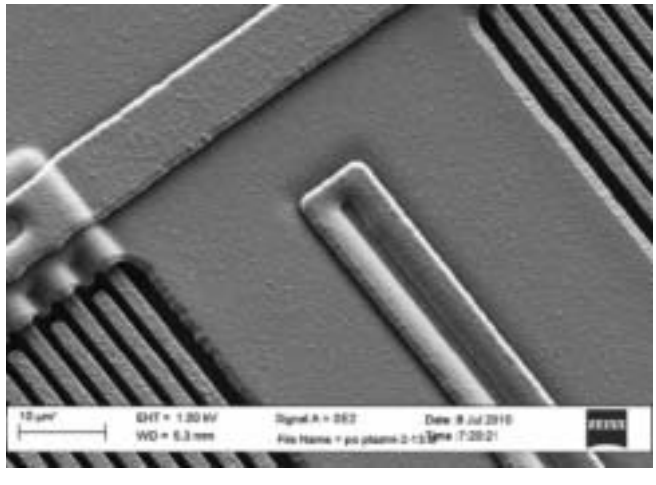

Figure 1: SEM of differential COMB sensor

In reality the detection level must be at least 100 times better than defined by equation (1) because the sensor is not modified 100%, adsorption/desorption is a dynamic process, so not all molecular traps are occupied at the same time and we want to detect minimum possible number of molecules in the air. From this short explanation of the sensor behavior it is clear that ultimate sensitivity is required from the electronics to be able to detect ppb level of selected molecules in the air.

Architecture and noise

To achieve required sensitivity using CMOS electronics at low power consumption many possible solutions exist (1,3,4). All of them are from the noise point of view inferior to the lock-in amplifier principle (5), which is used in this work. Signal processing is divided into analog signal processing (ASP) presented on Figure 2 and digital signal processing (DSP) presented on Figure 3. ASP is used to amplify weak signals coming from the sensor, to perform frequency shaping, down-mixing, filtering and A/D conversion. The DSP performs signal processing in digital domain: decimation filtering, down-mixing, averaging the result and taking care of the coordination. Very high 1/f noise corner frequency of modern MOS transistors require HF operation of the ASP to reach ultimate noise performances. This is achieved by driving differential sensor capacitors by high frequency square-wave signals V_{sp} and V_{sn} with adjustable amplitude A and frequency f_s. The signal at the output of the charge amplifier V_{cho} (equation 3)) is proportional to the difference of both capacitors $\Delta C_1 = C_p - C_n$, amplitude of sensing signals A and is inversely proportional to C_f (feedback capacitance of the CHA) and $H_{CHA}(s)$, that is a HP signal transfer function of the charge amplifier.

$$V_{cho}(s) \cong A\frac{\Delta C_1}{C_f} H_{CHA}(s) \qquad (3)$$

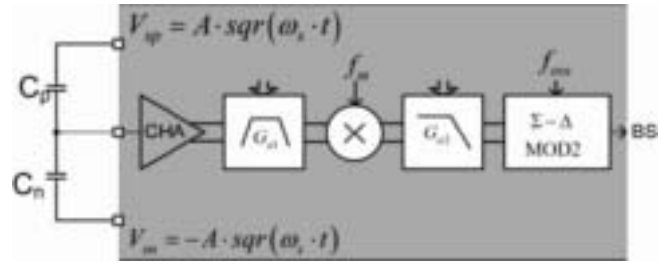

Figure 2: Architecture of the ASP circuit

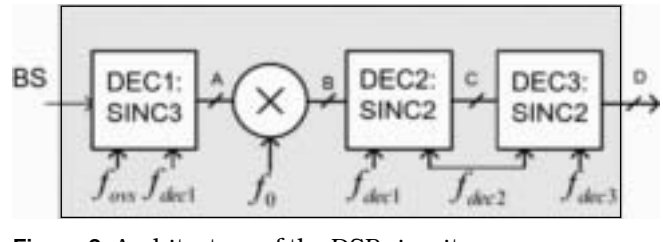

Figure 3: Architecture of the DSP circuit

Noise power density at the input of a first gain stage can be calculated using equation (4), where: $\sum C = 5pF$ that includes all capacitances connected to the virtual ground of CHA, $C_f = 2.1pF$ is a feedback capacitor of the CHA, $C_0 = 0.5pF$ is sensor capacitance and $R_f \cong 50M\Omega$ is the feedback resistor of the CHA. $V_{ndop} = 12nV/\sqrt{Hz}$ is input-referred noise density of the CHA amplifier and of the first gain stage. The noise contributions of sensing generators are negligible because $C_0 \le C_f$. The resulting noise at the input of first gain stage is approx. $V_{ndCHO} = 85nV/\sqrt{Hz}$ and the signal to noise ratio in 1Hz bandwidth for $\Delta C = 1aF$ is $SnR = 12dB$.

$$P_{ndG1\_in} \cong \left[V_{ndOP}\left(1+\frac{\sum C}{C_f}\right)\right]^2 + V_{ndG1}^2 + 2\cdot\left[V_{ndS}\left(\frac{C_0}{C_f}\right)\right]^2 + kTR_f \cdot H_{rf}^2(\omega_s) \quad (4)$$

The spectrum at the output of a charge amplifier including all noise sources is presented on Figure 4. The signal is BP filtered and amplified using low noise programmable gain amplifier with gain $G_{a1} = 11$. To maintain linearity of passive mixer and the following stages G_{a1} is limited because ΔC_0 is big.

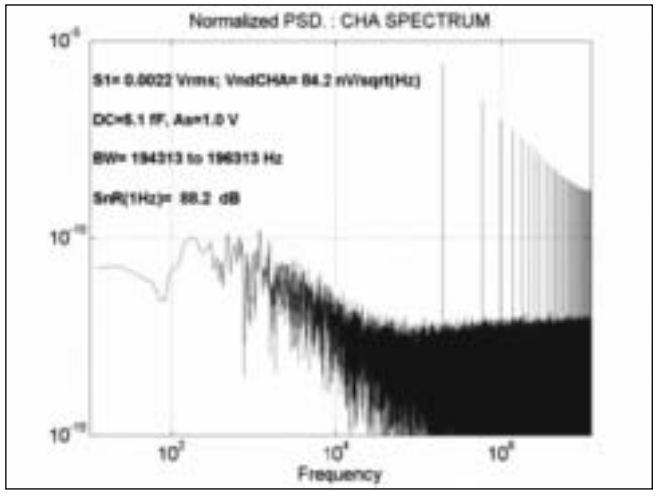

Figure 4: Output spectrum of CHA for $\Delta C = 5\,fF$

Down mixing reduces the frequency to $f_0 = f_s - f_m$ in the region between $f_{knee} < f_0 < f_{Q\,\mathrm{mod}}$, where f_{knee} is the corner frequency of the 1/f noise of CHA and $f_{Q\,\mathrm{mod}}$ is the corner frequency of the modulator shaped quantization noise. The parameters used are:, $f_s = 195.3kHz$ and $f_m = 192.3kHz$. The spectrum at the output of analog passive mixer (Figure 5) is limited by 2nd order continuous time LP filter to reduce out of band components before further amplification by $G_{a2} = 10$. After second gain stage the level is appropriate for A/D conversion using 2nd order ΣΔ modulator. The output of this conversion process is a bit-stream with spectrum presented on Figure 6. The main noise contribution around 3kHz spectral line comes from amplified charge amplifier noise.

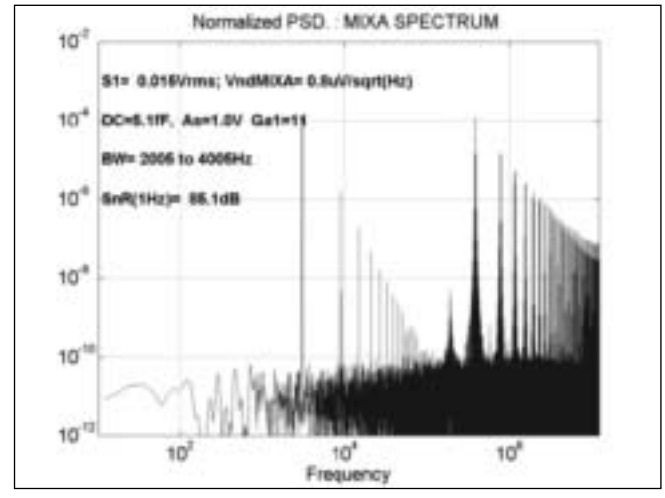

Figure 5: Spectrum at the output of passive mixer for $A = 1V$ and $\Delta C = 5\,fF$ $G_{a1} = 11$

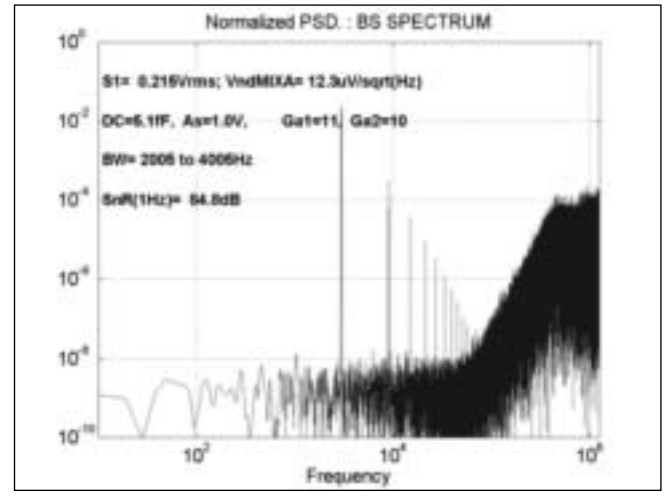

Figure 6: Spectrum at the output of the modulator (BS)

In DSP the BS is first filtered by DEC1 with $R_1 = 32$, $N_{dec1} = 3$ and $WL1 = 16$ that attenuates shaped out of band quantization noise and remains of HF spectral components from MIXA and reduces the sampling frequency down-to $f_{dec1} = 78kHz$. Figure 7 shows the spectrum. 16 bit digital stream is now composed of amplified 3kHz sine-wave with amplitude proportional to ΔC and amplified noise from the CHA. This signal is further mixed by digital mixer driven by square-wave with frequency $f_0 = f_s - f_m$. The result is 16 bit digital signal composed of DC compo-

nent that carries the information, HF spectrum and noise (Figure 8). Further filtering and decimation is performed by DEC2 and DEC3 with the following settings: $R_2 = 520,\ N_{dec2} = 2,\ WL_2 = 32,\ f_{dec2} = 150Hz$

$R_3 = 16,\ N_{dec3} = 2,\ WL_3 = 36,\ f_{dec3} = 9.3Hz,\ WL_{3out} = 24.$ The response to $\Delta C_0 = 5.1fF$ due to manufactured capacitors difference and the effect of one layer of adsorbed molecules ($\delta d = 0.1nm$) is shown on Figure 9 as a time domain response at the output of DEC3.

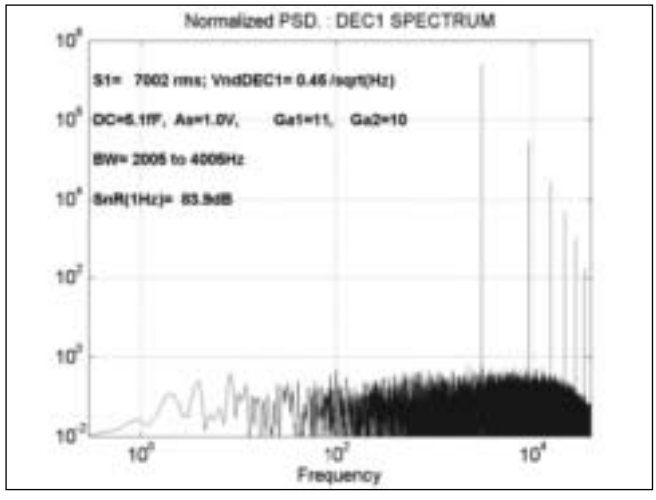

Figure 7: Spectrum at the output of the DEC1

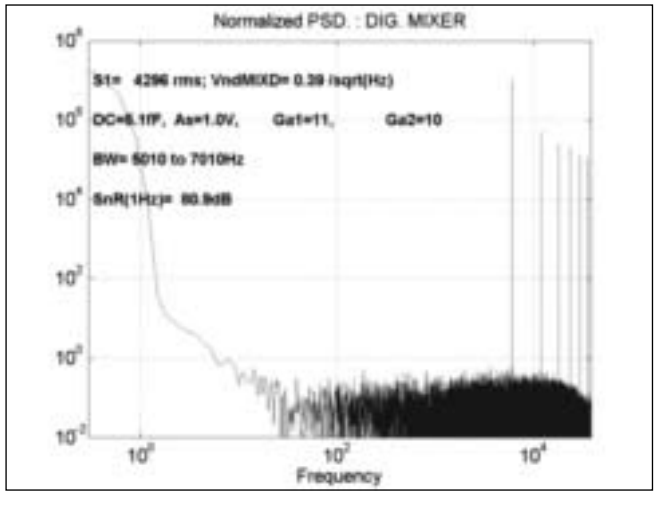

Figure 8: Spectrum after digital mixer

The digital signal is transferred via USB to the PC and presented on the screen in real time (6).

Modeling and measurements

Complete measurement system is modeled in Simulink before implementation. All important parameters as well as non-ideal effects of the ASP

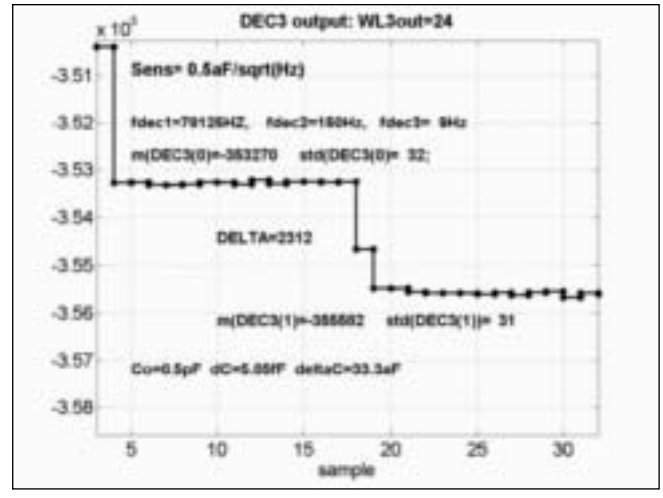

Figure 9: Response at DEC3 output to $\Delta C_0 = 5\,fF$ and $\delta C = 33aF$

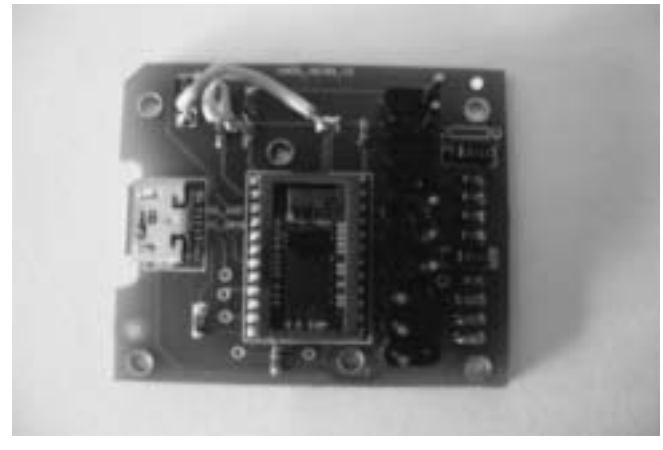

Figure 10: PCB with sensors and ASIC

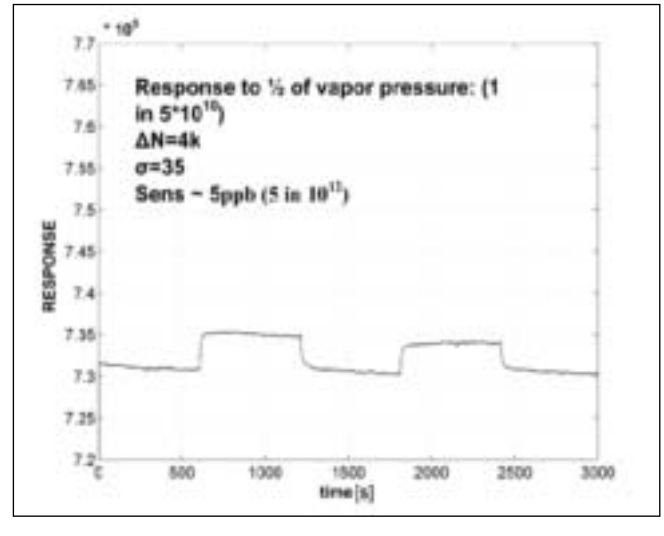

Figure 11: Measured response of the sensor

are included in the model like: GBW, offset voltages, slew-rates, noise power densities (thermal, 1/f, kT/C). At the same time the DSP is modeled as a bit-true model that can be used to compare simulation results with VHDL simulation results. The sensor is modeled as network of capacitors including parasitic and adsorption is modeled as a time and concentration dependent capacitor. Achieved sensitivity is below $0.5aF/\sqrt{Hz}$ that is proven by system level simulations and measurements with the demonstrator. Figure 10 shows a PCB with ASIC and sensors as a system in package (SiP). The response to $^1/_2$ of vapor pressure (the density 0.2 10^{-10}) is shown on Figure 11.

Conclusions

In this work the architecture, design, modeling and implementation of low-noise signal processing electronics needed to implement high-resolution MEMS/NEMS based capacitive sensors interface is described. Using double-mixing lock-in amplifier principle it is possible to sense a capacitance difference smaller than 0.5aF/√Hz on 2.1pF feedback capacitor. The chip is implemented in 0.35um BCD technology with approx. silicon area of 2.5mm^2. Two orders of magnitude improvements are still possible if sensors are implemented on top of the CMOS ASIC (reduced parasitic capacitances), noise of the CHA and gain stages are reduced 5 times and sensing voltages are increased 5 times. Because of achieved characteristics it is possible to use proposed interface for sensing capacitance changes of micro/nano scale chemical or biological sensors with ultimate sensitivity. The proposed improvements are currently in development.

References

1. Zhu Y, Joshua EY, Lee A, Seshia A. A resonant micromachined electrostatic charge sensor. IEEE Sensors J 2008; 8(9): 1499–505.

2. Strle D, Kempe V. MEMS based inertial systems. Informacije MIDEM 2007; 37(4): 199–209.

3. Peng SY, Qureshi MS, Hasler PE, Basu A, Degertekin FL. A charge-based low-power high-SNR capacitive sensing interface circuit. IEEE Trans Circ Syst I 2008; 5(7): 1863–72.

4. Enz C, Themes G. Circuit techniques for reducing the effects of OP-amp imperfections: auto-zeroing, correlated double sampling, and chopper stabilization. Proc IEEE 1996; 84(11): 1584–614.

5. Blair DP, Sydenham PH. Phase sensitive detection as a means to recover signals buried in noise. J Phys E: Sci Instrum 1975; 18(8): 621–7.

6. Nahtigal U, Strle D. Precision measurements of capacitance. In: Proceedings of the 45th International Conference on Microelectronics, Devices and Materials. Postojna, 2009: 371–4.

7. Joo S, Brown RB. Chemical sensors with integrated electronics. Chem Rev 2008; 108(2): 638–51.

NIZKOŠUMNO PROCESIRANJE SIGNALOV ZA MEMS/NEMS BIOLOŠKE SENZORJE: SISTEMSKI POGLED

Strle D.

Povzetek: Prispevek obravnava arhitekturo, modeliranje in implementacijo nizkošumnega elektronskega vezja, ki je uporabno za realizacijo visokoločljivega vmesnika med biološkimi in kemičnimi senzorji, ki temeljijo na tehnologiji MEMS/NEMS in elektronike ob pomoči osebnega računalnika. Uporabljen je princip ojačevalnika »lock-in« s posebnim dvojnim mešanjem, ki je optimalno porazdeljeno med vezjem za procesiranje signalov, in sicer analogno (ASP), in digitalno (DSP). Vezje je sestavljeno iz nizkošumnega ojačevalnika nabojev (CHA), programobilne ojačevalne stopnje, več različnih elektronskih filtrov, analognega mešalnika, ΣΔ analogno-digitalnega pretvornika in ustreznega DSP procesiranja. S temi elementi in takšno arhitekturo je mogoče doseči veliko razmerje signal/šum (več kot 134 dB) ter ločljivost, ki je v razredu 0.5 aF/√Hz pri senzorju s kapaciteto 2pF. Zaradi naštetih karakteristik je mogoče takšen elektronski vmesnik uporabiti kot vmesnik za zaznavo kapacitivnih sprememb pri miniaturnih mikro/nano kemičnih in bioloških senzorjih z veliko občutljivostjo. Vezje je implementirano v 0,35 µm tehnologiji CMOS in zavzema 2,5 mm^2. Izjemna občutljivost kapacitivnega senzorja in opisane elektronike omogoča zaznavo 5 ppb molekul v zraku pri sobni temperaturi.

Ključne besede: elektronski vmesnik za biološke senzorje; šum pri elektronskih vmesnikih; senzorski vmesniki za kapacitivne senzorje; ojačevalniki “lock-in”.

Slov Vet Res 2010; 47 (4): 155-8
UDC 57.08:591.46:612.4:599.323.4

Review Article

BEHAVIORAL CHARACTERIZATION OF STEROIDOGENIC FACTOR-1 KNOCKOUT MICE

Neža Grgurevič[1*], Stuart A. Tobet[2], Gregor Majdič[1,3]

[1]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; [2]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523-1680, USA; [3]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

*Corresponding author, E-mail: neza.grgurevic@gmail.com

Summary: Sex differences in the brain are mainly caused by sex steroid hormones. Steroidogenic factor-1 is a key regulator of gonadal and adrenal development, and SF-1 knockout mice (SF-1KO) are born without gonads and adrenal glands and the region of the ventromedial nucleus of the hypothalamus (VMH) is reorganized. Due to the absence of gonadal hormones during embryonic development and alterations of the VMH, SF-1 KO mice provide an important model to study VMH dependent and hormonally independent sex differences in brain and behaviour. Initial studies did not show significant sex differences that could be attributed to sex chromosome effects, but provided interesting differences in aggressive and affiliative behaviours between genotypes, that may be attributable to the disrupted cellular organization of the VMH in SF-1 KO mice. However, further more focused behavioural studies also revealed some sex differences that could be also attributed to the action of sex chromosomes.

Key words: brain; sex differences; mouse; behaviour

Introduction

Differences in the brains between males and females have been observed in many levels of structure and function (1). One of the central questions is how and when these differences arise? Mammalian sexual differentiation begins with an expression of the *Sry* gene normally found on the Y chromosome and differentiation of the male gonads. After testes develop, circulating testosterone (T) leads to long-lasting changes in the structure of the male brain. It is now generally accepted that in rodents, the process of masculinization and defeminization is mediated by estradiol, derived from the local aromatization of T. In females, ovaries do not secrete significant levels of steroid hormones during prenatal development and many female brain characteristics develop in the absence of hormonal secretion (2). However, some studies suggest that development of female brain also requires active feminization and this process could be regulated by estradiol secreted from the ovaries between birth and puberty (3). Later in life, gonadal secretion of steroid hormones continues to induce less permanent sex differences via activational effects. These differences are sex-specific caused by ovarian and testicular secretions and could be eliminated by gonadectomy (2). In the last 10-15 years, a growing number of studies have shown that some sex differences in the brain could arise independently of gonadal hormones and putatively by different effects of genes, especially located on the X or Y chromosomes (4). Such sex differences, in mice, have been described in parental and aggressive behaviour (5), learning of habits (6), and sniffing and grooming of an intruder (7). In most behavioural studies it is difficult to distinguish between organizational and activational effects since gonadally intact mice are used (4). Therefore we used a novel model, agonadal mice with disruption of the gene coding for steroidogenic factor-1. Steroidogenic factor 1 (SF-1), officially designated NR5A, was initially discovered as a regulator of the cytochrome P450 steroidogenic enzymes (8). Subsequent studies

Received: 13 September 2010
Accepted for publication: 15 November 2010

have defined broader roles for SF-1 in development and function of the hypothalamus-pituitary-gonadal axis. SF-1 KO mice are born without gonads and adrenal glands and have male to female sex reversal of secondary sex structures. The organization of the region that would normally contain the ventromedial nucleus of the hypothalamus (VMH) and gene expression in pituitary gonadotropes is also markedly altered in SF-1 KO mice (9). After neonatal corticosteroid injections and adrenal transplantation these mice can be studied in adulthood (10). Due to lack of gonadal hormones during embryonic development and alterations of the VMH SF-1 KO mice provide an important tool for delineating the roles of gonadal hormones and the VMH in a variety of sex dependent aspects of physiology and behaviour.

Behavioural analyses of SF-1 knockout mice

In a recent study SF-1 KO (genetically females and males) and WT mice were gonadectomized prior to puberty and tested for social behaviours. In these studies, no sex differences were found between chromosomally male and female (XX and XY) SF-1 KO mice in three different hormonal settings – in hormonally naïve mice and in mice primed with either testosterone or estradiol and progesterone. Nevertheless, these studies provided interesting results with regard to aggression in both hormonally naïve and testosterone treated SF-1 KO mice. Hormonally naïve SF-1 KO mice, in particular females, were aggressive against intruder mice (11). However, this aggression was moderate aggression and could be possibly attributed to increased anxiety like behaviour in SF-1 KO mice that was also found in other testing paradigms (e.g., EPM, (12)) and another study using VMH specific SF-1 knockout mice model (13). Interestingly, testosterone treatment reduced the aggression shown by SF-1 KO mice and induced strong aggressive behaviour only in WT male mice (11). This confirmed that testosterone during a developmental period is needed to display proper intermale aggressive behaviour as reported previously (14). Aromatization of T could be the major factor responsible for development of adult intermale aggression since ERαKO mice rarely display aggressive behaviour against bulbectomized males (15). In contrast, ERβKO (16) and testicularly feminized mice (17) do show normal intermale aggression.

In contrast to data for rats (18), our studies have shown that neonatal hormonal exposure is not necessary for proper expression of sex related behaviors in mice since both testosterone and estradiol/progesterone primed SF-1 KO mice display both male and female sex behaviour, respectively. Although lordosis quality was not scored in our initial studies with EB+P primed mice, we can speculate from the copulatory behaviour of male mice that SF-1 KO mice were less receptive than WT females and also that females were more receptive than males regardless of their genotype, and this was further confirmed in follow up studies (19). Lower receptivity of SF-1 KO mice could be due to the absence of gonadal hormones during development (3), or just as likely due to the altered organization of the VMH. It is well known that the VMH plays important role(s) in lordosis behaviour and, consistent with our findings, impaired lordosis was also found in CNS-specific SF-1 KO mice (20). So far it is not known if genetic factors contribute to defeminization of female sexual behaviour in males independently or in concert with gonadal hormones. Defeminization could be regulated through ERβ, since higher lordosis quotient was observed in ERβKO males compared to the WT males (21). It would be interesting to establish whether SF-1 KO males are partially defeminized and if this process is regulated through different Y linked genes, ERβ, or dopaminergic systems since expression of tyrosine hydroxylase is regulated by the *Sry* gene in the midbrain (22). Further experiments will be needed to test these hypotheses.

Acknowledgements

This work was supported by NIH grant MH61376 (S.A.T. and G.M.), ICGEB grant CRP SLO 06/02, ARRS (Slovenian research agency) grants P4-0053 and J7-2093 (G.M.).

References

1. Cooke B, Hegstrom CD, Villeneuve LS, et al. Sexual differentiation of the vertebrate brain: principles and mechanisms. Front Neuroendocrinol 1998; 19(4): 323–62.

2. MacLusky NJ, Naftolin F. Sexual differentiation of the central nervous system. Science 1981; 211(4488): 1294–302.

3. Bakker J, Baum MJ. Role for estradiol in female-typical brain and behavioral sexual differentiation. Front Neuroendocrinol 2008; 29(1): 1–16.

4. Arnold AP. Mouse models for evaluating sex chromosome effects that cause sex differences in non-gonadal tissues. J Neuroendocrinol 2009; 21(4): 377–86.

5. Gatewood JD, Wills A, Shetty S, et al. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. J Neurosci 2006; 26(8): 2335–42.

6. Quinn JJ, Hitchcott PK, Umeda EA, et al. Sex chromosome complement regulates habit formation. Nat Neurosci 2007; 10(11): 1398–400.

7. McPhie-Lalmansingh AA, Tejada LD, Weaver JL, et al. Sex chromosome complement affects social interactions in mice. Horm Behav 2008; 54(4): 565–70.

8. Lala DS, Rice DA, Parker KL. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. Mol Endocrinol 1992; 6(8): 1249–58.

9. Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 1997; 18(3): 361–77.

10. Majdic G, Young M, Gomez-Sanchez E, et al. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002; 143(2): 607–14.

11. Grgurevic N, Budefeld T, Rissman E F, et al. Aggressive behaviors in adult SF-1 knockout mice that are not exposed to gonadal steroids during development. Behav Neurosci 2008; 122(4): 876–84.

12. Grgurevic N, Tobet SA, Rissman EF, Majdic G. SF-1 knockout mice – a novel model for behavioral studies from perspective of sex steroids and VMH structure. In: Society for behavioral neuroendocrinology annual meeting. Pacific Grove, USA, 2007.

13. Zhao L, Kim K W, Ikeda Y, et al. Central nervous system-specific knockout of steroidogenic factor 1 results in increased anxiety-like behavior. Mol Endocrinol 2008; 22(6): 1403–15.

14. vom Saal F S. Prenatal exposure to androgen influences morphology and aggressive behavior of male and female mice. Horm Behav 1979; 12(1): 1–11.

15. Ogawa S, Lubahn D B, Korach K S, et al. Behavioral effects of estrogen receptor gene disruption in male mice. Proc Natl Acad Sci U S A 1997; 94(4): 1476–81.

16. Ogawa S, Chan J, Chester A E, et al. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc Natl Acad Sci U S A 1999; 96(22): 12887–92.

17. Scordalakes E M, Rissman E F. Aggression and arginine vasopressin immunoreactivity regulation by androgen receptor and estrogen receptor alpha. Genes Brain Behav 2004; 3(1): 20–6.

18. Bonthuis P J, Cox K H, Searcy B T, et al. Of mice and rats: key species variations in the sexual differentiation of brain and behavior. Front Neuroendocrinol 2010; 31(3): 341–58.

19. Grgurevic N, Rissman EF, Tobet SA, Majdic G. Differentiation of sexual behavior potential in agonadal steroidogenic factor 1 knockout mice. In: Society for behavioral neuroendocrinology annual meeting. Groeningen, Netherlands, 2008.

20. Kim K W, Li S, Zhao H, et al. CNS-specific ablation of steroidogenic factor 1 results in impaired female reproductive function. Mol Endocrinol 2010; 24(6): 1240–50.

21. Kudwa AE, Michopoulos V, Gatewood JD, et al. Roles of estrogen receptors alpha and beta in differentiation of mouse sexual behavior. Neuroscience 2006; 138(3): 921-8.

22. Dewing P, Chiang CW, Sinchak K, et al. Direct regulation of adult brain function by the male-specific factor SRY. Curr Biol 2006; 16(4): 415–20.

PROUČEVANJE OBNAŠANJA MIŠI BREZ GENA SF-1

N. Grgurevič, S.A. Tobet, G. Majdič

Povzetek: Spolne razlike v možganih nastanejo predvsem zaradi delovanja spolnih hormonov. Steroidogeni faktor 1 (SF-1) je dejavnik, ki uravnava razvoj spolnih žlez in nadledvične žleze. Miši brez gena SF-1 (angl. Knockout; SF-1 KO) se rodijo brez spolnih in nadledvičnih žlez in imajo spremenjeno strukturo ventromedialnega jedra hipotalamusa (VMH). Zaradi pomanjkanja spolnih hormonov med embrionalnim življenjem in zaradi spremenjenega jedra VMH predstavljajo miši SF-1 KO pomemben model za proučevanje hormonsko neodvisnih in od VMH odvisnih spolnih razlik v možganih in v obnašanju. Naše začetne raziskave niso pokazale spolnih razlik, ki bi jih lahko pripisali vplivu spolnih kromosomov, vendar pa so pokazale zanimive razlike v agresivnem in socialnem obnašanju med kontrolnimi mišmi in mišmi brez gena SF-1, ki bi jih lahko pripisali spremenjeni strukturi jedra VMH. Pri bolj usmerjenih raziskavah v določene tipe obnašanja pa smo ugotovili nekatere zanimive razlike med spoloma tudi pri miših brez gena SF-1, ki kažejo na vpliv spolnih kromosomov na spolno različen razvoj možganov.

Ključne besede: možgani; spolne razlike;miš; obnašanje

Slov Vet Res 2010; 47 (4): 159-62
UDC 575.825:316.6:591.5:599.323.45

Review Article

SOCIAL ISOLATION DURING PUBERTY AFFECTS SOCIAL BEHAVIOUR IN ADULT MICE

Jasmina Kerčmar[1]*, Gregor Majdič[1,2]

[1]Center for Animal Genomics, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana; [2]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

*Corresponding author, E-mail: jasmina.kercmar@vf.uni-lj.si

Summary: Early social isolation can have profound consequences on different social behaviours due to alterations in brain structures or gene expressions, but its influence on social recognition or vasopressin (AVP) and oxytocin (OXT) expression has not been thoroughly investigated in mice. We examined social recognition in mice of both sexes that were individually housed from 30 days of age until testing at around day 80, individually housed from day 30 until day 60 and regrouped from day 60 until testing at day 80 and in control mice that were group housed throughout experiment. The ability to recognize familiar mouse was tested using standard social recognition test. Group housed mice showed strong social memory, whereas individually housed did not. Interestingly, mice reared in isolation for a limited period showed reduced social memory, suggesting that even isolation for a limited period can have lasting behavioural deficit, especially in female mice. Using immunohistochemistry we examined vasopressin and oxytocin expression in the brain. As expected, immunohystochemical detection of AVP in lateral septum (LS) revealed robust sex difference with males having much more AVP in fibers than females. However, there were no obvious differences in either vasopressin or oxytocin between groups in different housing regimes, suggesting that social isolation in mice has no effect on the expression of these two neurohormones.

Key words: mice; social stress; isolation; social behaviour; social recognition; vasopressin; oxytocin

Introduction

In the natural conditions, mouse *(Mus musculus)* is a social species living in large social groups establishing group territories (1)**.** The ability to recognize familiar conspecifics, social recognition memory, is critical for many forms of social interactions (2). But in laboratory conditions they are often individually housed to prevent intermale aggression or unwanted matings (1, 3). Many studies have shown that early social deprivation, not only in rodents but also in primates and humans, can induce different behavioural, brain structure and gene expression abnormalities (4, 5). It can cause hyperactivity, reduction in habituation and reduction in anxiety-like behaviour in the elevated plus maze (EPM) test, but an opposite effect in the dark-light (3) and staircase test (6), impairment in novel object recognition (7), aberrant self-manipulation, frequent chasing and biting of the tail (1) and higher levels of aggressive attacks in males (8).

Isolation in rats induces enlargements in different stress-sensitive brain regions (5), cytoskeletal microtubular alterations in the hippocampus (9) and reduction in the size of medial prefrontal cortex (10). It also alters peripheral vasopressin (AVP) and oxytocin (OXT) concentrations, and a lack of social stimuli adversely affects development of these two systems in rats (11).

Social isolation and social recognition

Social recognition in rodents is critical for the formation and maintenance of all social relationships. The influence of social isolation on performance in social recognition tests has not been thoroughly investigated. There are only two studies that reported impairments in social recognition in individually housed male (12) and female rats (11). Our

Received: 15 September 2010
Accepted for publication: 17 November 2010

study revealed that the strongest pattern of social recognition is present in socially housed males. Social recognition was also observed in socially female mice with much smaller reduction in sniffing time (lower habituation), but still with significant difference between last two trials (the last trial with a new unfamiliar female), suggesting that they could distinguish familiar from unfamiliar mouse. In contrast, both male and female mice that were isolated throughout the test did not show either habituation during the first 8 tests and neither social recognition as there was no significant difference between tests 8 and 9. In male mice isolated for a limited period the habituation was reduced, although social recognition was still present as evident by significant difference between tests 8 and 9. However, in female mice that were isolated for a limited period, there was no social recognition (although habituation was similar to social female mice), suggesting that even isolation for a limited period can have lasting effect on this behaviour (13).

Social isolation and expression of AVP and OXT

Social isolation has been reported to affect expression of hypothalamic OXT and AVP (11), which are important in modulating the social recognition and other social behaviours (reviewed in (14, 15). Lateral septum, medial amygdala (MeA), hippocampus, hypothalamus, olfactory bulbs and vomeronasal organ have all been demonstrated as regions critical for OXT and AVP effects on social recognition (16). Previous studies have shown that administration of AVP agonists into LS have improved (17), while AVP antagonists have blocked normal social recognition in rats (18). Post-weaning social isolation can decrease number of AVP cells in male or OXT in female rats in the paraventricular nucleus (PVN), what coincides with the impairment in social recognition in isolated rats (11) and with the suggestion that AVP is more important in male (14), and OXT in female behaviour (15).

In our study, immunoexpression of AVP in LS, which contains axons from the MeA and bed nucleus of the stria terminalis (BNST), and OXT in PVN was not altered by social isolation. However, since we only used immunocytochemistry that could only detect proteins stored in the nerve fibers, it is still possible that there are differences in either of these two peptides at the level of protein secretion or turnover, or even at the level of their receptors expression, therefore, we were not able to either confirm or reject the hypothesis that dysregulation of AVP and/or OXT system in the brain is responsible for alterations in social recognition behaviour in socially isolated mice (16).

Acknowledgements

This work was supported by ICGEB grant CRP SLO 06/02 and ARRS (Slovenian research agency) grants P4-0053 and J7-2093.

References

1. Crawley JN. What´s wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice. New York: Wiley- Liss, 2000: 167–77.

2. Markham JA, Juraska JM. Social recognition memory: influence of age, sex, and ovarian hormonal status. Physiol Behav 2007; 92(5): 881–8.

3. Võikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. Genes Brain Behav 2005; 4(4): 240–52.

4. Agis-Balboa RC, Pinna G, Pibiri F, et al. Down-regulation of neurosteroid biosynthesis in corticolimbic circuits mediates social isolation-induced behavior in mice. Proc Natl Acad Sci U S A 2007; 104(47): 18736–41.

5. Spinelli S, Chefer S, Suomi SJ, et al. Early-life stress induces long-term morphologic changes in primate brain. Arch Gen Psychiatry 2009; 66(6): 658–65.

6. Ago Y, Takahashi K, Nakamura S, et al. Anxiety-like and exploratory behaviors of isolation-reared mice in the staircase test. J Pharmacol Sci 2007; 104(2): 153–8.

7. Heidbreder CA, Weiss IC, Domeney AM, et al. Behavioral, neurochemical and endocrinological characterization of the early social isolation syndrome. Neuroscience 2000; 100(4): 749–68.

8. Wongwitdecha N, Marsden CA. Social isolation increases aggressive behaviour and alters the effects of diazepam in the rat social interaction test. Behav Brain Res 1996; 75(1/2): 27–32.

9. Bianchi M, Fone KF, Azmi N, et al. Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. Eur J Neurosci 2006; 24(10): 2894–902.

10. Schubert MI, Porkess MV, Dashdori N, Fone KC, Auer DP. Effects of social isolation rearing on the limbic brain: a combined behavioral and mag-

netic resonance imaging volumetry study in rats. Neuroscience 2009; 159(1): 21–30.

11. Tanaka K, Osako Y, Yuri K. Juvenile social experience regulates central neuropeptides relevant to emotional and social behaviors. Neuroscience 2010; 166(4): 1036–42.

12. Zhao X, Sun L, Jia H, et al. Isolation rearing induces social and emotional function abnormalities and alters glutamate and neurodevelopment-related gene expression in rats. Prog Neuropsychopharmacol Biol Psychiatry 2009; 33(7): 1173–7.

13. Kercmar J, Budefeld T, Grgurevic N, Tobet SA, Maidic G. Adolescent social isolation changes social recognition in adult mice. Behav Brain Res 2010, in press doi:10.1016/j.bbr.2010.09.007.

14. Caldwell HK, Lee HJ, Macbeth AH, Young Ill WS. Vasopressin: Behavioral roles of an ˝original˝ neuropeptide. Prog Neurobiol 2008; 84(1): 1–24.

15. Neumann ID. Brain oxytocin: A key regulator of emotional and social behaviours in both females and males. J Neuroendocrinol 2008; 20(6): 858–65.

16. Bielsky IF, Young LJ. Oxytocin, vasopressin, and social recognition in mammals. Peptides 2004; 25(9): 1565–74.

17. Engelmann M, Ludwig M, Landgraf R. Simultaneous monitoring of intracerebral release and behavior: vasopressin improves social recognition. J Neuroendocrinol 1994; 6(4): 391–5.

18. Everts HGJ, Koolhaas JM. Differential modulation of lateral septal vasopressin receptor blockade in spatial-learning, social recognition, and anxiety-related behaviors in rats. Behav Brain Res 1999; 99(1): 7–16.

SOCIALNA OSAMITEV MED PUBERTETO VPLIVA NA SOCIALNO OBNAŠANJE PRI ODRASLIH MIŠIH

J. Kerčmar, G. Majdič

Povzetek: Zgodnja socialna osamitev, ki povzroča spremembe tako v strukturi možganov kot tudi v izražanju genov, ima lahko pomemben vpliv na različna socialna obnašanja. Vpliv osamitve pri miših na socialno prepoznavanje ali izražanje vazopresina (AVP) in oksitocina (OXT) pa še ni bil raziskan. Proučevali smo socialno prepoznavanje miši obeh spolov, ki so bile nastanjene individualno vse od starosti 30 dni pa do testiranja pri starosti 80 dni, individualno nastanjene od 30. do 60. dneva in nato od 60. dneva ponovno skupinsko nastanjene in kontrolna skupina, ki je bila cel čas nastanjena skupinsko. S standardnim testom socialnega prepoznavanja smo ugotavljali sposobnost testnih miši ločiti znano miš od neznane. Skupinsko nastanjene miši so kazale neokrnjen, močan socialni spomin, medtem ko ga miši, nastanjene individualno, niso. Zanimivo je, da so individualno nastanjene miši za določeno časovno obdobje, kazale slabši socialni spomin, kar pomeni, da ima lahko tudi osamitev za določen čas trajne posledice pri socialnem obnašanju, še posebej pri mišjih samicah. Z imunohistokemično metodo smo ugotavljali izražanje vazopresina in oksitocina v možganih. Po pričakovanjih smo našli očitno spolno razliko v izražanju vazopresina v stranskem septumu (LS). Samci so imeli namreč več vazopresina v živčnih vlaknih kot samice. V izražanju tako vazopresina kot oksitocina glede na način nastanitve nismo našli razlik.

Ključne besede: miši; socialni stres, osamitev; socialno prepoznavanje; vazopresin; oksitocin

Slov Vet Res 2010; 47 (4): 163-6
UDC 502/504:615

Review Article

PESTICIDES AS ENDOCRINE DISRUPTORS

Katerina Čeh[1]*, Gregor Majdič[1,2]

[1]Center for Animal Genomics, Veterinary Faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana; [2]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

*Corresponding author, E-mail: katerina.ceh@vf.uni-lj.si

Summary: Several synthetic chemicals used as pesticides have the capacity to interfere with hormone action in the mammalian body. These chemicals are known as endocrine disruptors. Exposure to endocrine disruptors before birth can change the development of sexual organs, neuroendocrine system and behaviour. We are studying whether long term exposure to low doses of organophosphorus insecticide Chlormephos and herbicide Atrazine affects development and function of reproductive tract and brain. In Chlormephos study, adult male and female mice were exposed to 3,5 μg/ml and 0,35 μg/ml of Chlormephos in the drinking water. No statistically significant differences between treated and control groups were found in any of the observed parameters that included several indicators of testis development and blood levels of reproductive hormones, suggesting that Chlormephos does not act as an endocrine disruptor in reproductive tract. Elevated plus maze test revealed increased anxiety like behaviour in mice exposed neonatally to higher dose of Chlormephos. Microarray analyses revealed some differences in expression of genes that might be involved in the anxiety-like behaviour but we could not confirm several of them using quantitative RT PCR. On the other hand, studies with Atrazine did reveal some endocrine effects of prenatal and neonatal exposure to Atrazine, although these studies are still on-going and the results are not conclusive, yet.

Key words: endocrine disruptors; chlormephos; atrazine; reproductive tract; brain

Introduction

Several synthetic chemicals used as pesticides or pharmaceutical agents can possibly act as endocrine disruptors (ED), ED have the capacity to interfere with hormone action in the mammalian body. Exposure to ED before birth can change the development of sexual organs, neuroendocrine system and behavior. ED have low hormone activity in comparison to endogenous hormones, but their ability to accumulate in the body fat and long half-life of some of them could increase their concentrations in the animal body. Environmental chemicals could act as estrogens, antiestrogens or antiandrogens. Different EDs do not have structural similarities and therefore it is not possible today to predict which chemical could act as an endocrine disruptor (1).

Organophosphorus compounds (OPs) are a large class of chemicals used for various purposes like chemical weapons, pesticides and antiparasitics. At high doses, OPs are irreversible inhibitors of acetylcholinesterase, causing accumulation of acetylcholine in cholinergic nervous system. However, little is known about possible toxic effects of exposure to low doses of organophosphorous compounds.(2). Atrazine is one of the most widely used herbicides and as such, a very common water pollutant. Most EU countries set the limit for Atrazine contamination of drinking water at 0.1 μg/L, but in areas with intensive farming this limit is often exceeded and could reach up to 1 μg/L. Several studies have shown that Atrazine could affect the endocrine system, primarily the hypothalamic-pituitary-gonadal axis (3, 4), although there is still controversy whether the Atrazine really is an endocrine disruptor.

Organophosphorous compounds and endocrine disruption

Several studies examined whether OP substances could act as endocrine disruptors. Oka-

Received: 17 September 2010
Accepted for publication: 18 November 2010

hashi et al.(5) reported that high doses of fenitrotion decrease activity of brain cholinesterase in exposed animals, but does not affect reproductive performance, organ weights, histopathology of testes, accessory sex organs, pituitary, thyroid, ovaries, uterus and sperm parameters in rats. In the same study, no general toxicity or effect on anogenital distance, retention of areolae, onset of puberty, organ weights, histopathological findings and sperm parameters were observed in the F1 generation. Similarly, in our unpublished study, Chlormephos did not affect number of pups in litters, daily sperm production, weight of testes and seminal vesicles , number of apoptotic cells and fertility. However, in contrast to that, Narayana et al. (6) reported that injection of methyl parathion in Wistar rats at doses that are relevant to human exposures (0,5 – 1 mg/kg) caused decrease in sperm count and increase in morphological defects in semen, although the number of pups in litters from treated animals did not differ from control groups, suggesting that effect of methyl parathion is still small. Some OPs in high concentrations do have direct effect on reproductive and endocrine system in humans (7) and animals (6). In our study, we did not find any difference in daily sperm production and number of apoptotical cells in the offspring of treated animals, but we did not examine these two parameters in treated mice so we do not know whether there are any direct effect of Chlormephos on sperm development, although even if present, such effects would be small as we did not find any differences in the litter sizes between treated and control groups.

Several reports demonstrated that exposure to bisphenol A and metoxychlor before birth and during early postnatal period could affects sexual and non-sexual behavior (8, 9). In our study we observed increased anxiety-like behavior in adult mice that were exposed to Chlormephos only during neonatal period. OPs are soluble in lipids so they can pass through blood-brain-barrier and can get into direct contact with developing nerve cells and could therefore directly impact development of nerve cells in the brain. On the other hand, several studies have shown that OPs could disrupt blood-brain barrier (BBB) in mice and rats with long term exposure to low doses, and such disruption of blood brain barrier could enable other harmful substances to enter the brain and cause delayed behavioral effects observed in our study (10, 11)

Atrazine as endocrine disruptor

Atrazine is one of the most commonly used herbicides in the world and poses special concern because it is ubiquitous, persistent contaminant of groundwater and surface water that is active at low, ecologically relevant concentrations (12). Studies showed that Atrazine can affect the reproductive system in animals such as frogs and rats (13, 14). However, almost all studies examining Atrazine effects in mammals used large doses of Atrazine, which could mimic an exposure of workers working with Atrazine, but are usually not a concern for the general population. Atrazine does not act in the same manner as classical endocrine disruptors that influence estrogen or androgen receptors (15) but possibly induces expression of the CYP19 gene (16). Some studies have shown that, at least in vitro, Atrazine could increase the activity of P450 aromatase and thus elevate estrogen production. Most studies about the effects of Atrazine were described in females, but some studies demonstrated that it can affect male reproductive system. One recent study have thus shown that *in utero* and through milk exposure to Atrazine affects development of prostate and seminal vesicles, suggesting that Atrazine can pass through the placenta and/or into milk and can affect offspring of treated mothers (17). In our study we examined whether exposure to Atrazine *in utero* and during early postnatal period at doses relevant for the general human population, could affect reproductive tract development in male mice. Preliminary results do suggest certain affects especially on the development of male reproductive tract such as reduced daily sperm production and increased number of apoptotic cell in testes from neonatally treated mice, although further studies are now being conducted to confirm preliminary findings.

References:

1. McLachlan JA. Environmental signaling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocr Rev 2001; 22(3): 319–41.

2. Weinbroum AA. Pathophysiological and clinical aspects of combat anticholinesterase poisoning. Br Med Bull 2004; 72: 119–33.

3. Villanueva CM, Durand G, Coutte MB, et al. Atrazine in municipal drinking water and risk of low birth weight, preterm delivery, and small-for-gesta-

tional-age status. Occup Environ Med 2005; 62(6): 400–5.

4. Cooper RL, Stoker TE, Tyrey L, et al. Atrazine disrupts the hypothalamic control of pituitary-ovarian function. Toxicol Sci 2000; 53(2): 297–307.

5. Okahashi N, Sano M, Miyata K, et al. Lack of evidence for endocrine disrupting effects in rats exposed to fenitrothion in utero and from weaning to maturation. Toxicology 2005; 206(1): 17–31.

6. Narayana K, Prashanthi N, Nayanatara A, et al. Effects of methyl parathion (o,o-dimethyl o-4-nitrophenyl phosphorothioate) on rat sperm morphology and sperm count, but not fertility, are associated with decreased ascorbic acid level in the testis. Mutat Res 2005; 588(1): 28–34.

7. Recio R, Ocampo-Gomez G, Moran-Martinez J, et al. Pesticide exposure alters follicle-stimulating hormone levels in Mexican agricultural workers. Environ Health Perspect 2005; 113(9): 1160–3.

8. Palanza P, Gioiosa L, vom Saal FS, et al. Effects of developmental exposure to bisphenol A on brain and behavior in mice. Environ Res 2008; 108(2): 150–7.

9. Panzica GC, Viglietti-Panzica C, Mura E, et al. Effects of xenoestrogens on the differentiation of behaviorally-relevant neural circuits. Front Neuroendocrinol 2007; 28(4): 179–200.

10. Song X, Pope C, Murthy R, et al. Interactive effects of paraoxon and pyridostigmine on blood-brain barrier integrity and cholinergic toxicity. Toxicol Sci 2004; 78(2): 241–7.

11. Gupta A, Agarwal R, Shukla GS. Functional impairment of blood-brain barrier following pesticide exposure during early development in rats. Hum Exp Toxicol 1999; 18(3): 174–9.

12. Solomon KR, Carr JA, Du Preez LH, et al. Effects of atrazine on fish, amphibians, and aquatic reptiles: a critical review. Crit Rev Toxicol 2008; 38(9): 721–72.

13. Cooper RL, Laws SC, Das PC, et al. Atrazine and reproductive function: mode and mechanism of action studies. Birth Defects Res B Dev Reprod Toxicol 2007; 80(2): 98–112.

14. Hayes TB, Collins A, Lee M, et al. Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci U S A 2002; 99(8): 5476–80.

15. Roberge M, Hakk H, Larsen G. Atrazine is a competitive inhibitor of phosphodiesterase but does not affect the estrogen receptor. Toxicol Lett 2004; 154(1-2): 61–8.

16. Sanderson JT, Letcher RJ, Heneweer M, et al. Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. Environ Health Perspect 2001; 109(10): 1027–31.

17. Rayner JL, Enoch RR, Wolf DC, et al. Atrazine-induced reproductive tract alterations after transplacental and/or lactational exposure in male Long-Evans rats. Toxicol Appl Pharmacol 2007; 218(3): 238–48.

PESTICIDI KOT HORMONSKI MOTILCI

K. Čeh, G. Majdič

Povzetek: Nekatere kemične snovi, ki jih uporabljamo kot pesticide, lahko motijo hormonsko ravnovesje v telesu. Imenujemo jih hormonski motilci. Izpostavljenost hormonskim motilcem pred rojstvom lahko vpliva na razvoj spolnih organov, na nevroendokrini razvoj in na obnašanje. V naši raziskavi proučujemo vpliv dolgotrajne izpostavljenosti nizkim dozam organofosfatnega insekticida klormefosa in pesticida atrazina na razvoj in delovanje spolih organov in možganov pri miših. Odraslim samcem in samicam smo smo v pitno vodo vmešali klormefos v koncentraciji 3,5 µg/ml in 0,35 µg/ml. Opazovali smo nekatere parametre dozorevanja mod in koncentracijo spolnih hormonov v krvi, vendar med poskusno in testnima skupinama nismo našli statistično značilnih razlik. Rezultati kažejo, da klormefos ne vpliva kot endokrini motilec na spolni sistem. Miši, ki so bile pred rojstvom in v zgodnjem poporodnem obdobju izpostavljene višji dozi klormefosa so v testu dvignjenega labirinta kazale povečano obnašanje podobno tesnobi. Analiza DNK mikromrež je pokazala razlike v izražanju nekaterih genov, ki so povezani z obnašanjem podobnim anksioznemu, vendar pa razlik nismo mogli potrdit z metodo kvantitativnega RT PCR. Raziskava izpostavljenosti miši nizkim dozam atrazina v obdobju pred in zgodaj po rojstvu je pokazala nekaj endokrinih vplivov, vendar pa rezultati še niso dokončni, saj raziskave še potekajo.

Ključne besede: hormonski motilci; klormefos; atrazin; spolni sistem; možgani

Slov Vet Res 2010; 47 (4): 167-70
UDC 577.25:612.4:599.323.4

Review Article

GONADAL HORMONE INDEPENDENT SEX DIFFERENCES IN STEROIDOGENIC FACTOR 1 KNOCKOUT MICE BRAIN

Tomaž Büdefeld[1*], Stuart A. Tobet[2], Gregor Majdič[1,3]

[1]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana, Slovenia; [2]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523-1680, USA; [3]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

*Corresponding author, E-mail: tomaz.budefeld@vf.uni-lj.si

Summary: Sex differences in brain morphology have been described in a number of species including humans. Gonadal hormones were shown to provide a major influence on brain sexual differentiation more than 50 years ago. A growing number of studies is providing evidence for roles of genetic factors, in particular sex chromosome complement, on brain sexual differentiation in mammals. In this review, hormone-independent brain sexual differentiation, with the emphasis on mice with a disruption of the SF-1 gene (SF-1 knockout, SF-1 KO) are discussed.

Keywords: brain sexual differentiation; sex chromosomes; SF-1 KO mice; preoptic area and hypothalamus; neuronal NO synthase; calbindin D-28k; neuropeptide Y

Introduction

Permanent effect of sex hormones on sex specific brain development was clearly described in 1959 when Phoenix and coworkers reported the organizational effect of prenatal exposure to exogenous testosterone on brain function in adult guinea pig females (1). Today it is widely accepted that gonadal steroid hormones are the major factors that shape brain development and function in a sex specific manner.

Male and female cells differ in their genetic blueprint consisting of autosomal and sex chromosomes. However, the direct effects of genes located on specific chromosomes on brain sexual differentiation are difficult to study due to the multiple factors contributing to sex specific brain structure and function. In the last decade, development of specific mouse genetic models has energized studies of direct effects of genetic factors on brain sexual development.

Received: 30 September 2010
Accepted for publication: 8 November 2010

Genetic factors in brain sexual development and function

Genes encoded on sex chromosomes are expressed in the brain where they could regulate expression of neural proteins, presumably in a sex specific manner. For example, *Sry* gene expression was found in tyrosine hydroxylase expressing neurons in the substantia nigra of adult mice and rats (2). However, the effects of genetic factors during brain sexual development are often masked by the actions of sex steroids.

One way to study genetic effects independently of steroid hormones is to study them in experimental systems before exposure to steroid hormones. In mice, gonadal primordia differentiate into testes or ovaries between embryonic day 10.5 and 12.5. Testes become steroidogenically active on embryonic day 13.0, while ovaries do not secrete steroid hormones until the first week after birth. Consequently, sex differences developed in the brain before gonadal differentiation must be caused by genetic factors. Genomic studies of brains from embryonic mice before or shortly after gonadal differentiation indeed found sex differences in gene expression of X and Y linked genes (3, 4) suggesting that sex chromo-

somes could have a role in brain sexual differentiation during early embryonic development. A study by Carruth and co-workers (5) showed sex differences in the expression of tyrosine hydroxylase in mesencephalic tissue slices dissected from the fetal mouse brain before gonadal development. A different approach to study genetic influences on brain sexual development is using animal models that are exposed to the same gonadal steroid hormones but differ in sex chromosome complements. Such are four core genotype (FCG) mice, developed by translocation of the *Sry* gene onto the autosome together with the deletion of the *Sry* from the Y chromosome (6). Comparisons of XX and XY gonadal males and XX and XY gonadal females showed the effect of sex chromosome complement on density of fibers expressing arginine vasopressin in the lateral septum (7) and on some behavioral traits (8).

SF-1 KO mice; an animal model for studying gonadal hormone independent brain sexual differentiation

Steroidogenic factor 1 (SF-1) is a transcription factor that regulates expression of a plethora of genes involved in development and function of endocrine organs. In mice with disruption of the *SF-1* gene (SF-1 knockout, SF-1 KO) gonadal and adrenal primordia regress early during development (9). The absence of adrenal glands makes the SF-1 KO genotype lethal after birth. With adrenal transplantation, SF-1 KO mice can be kept alive into adulthood (10) and since these mice are not exposed to the endogenous gonadal steroids, they represent a useful animal model for studying genetic and hormonal contributions to brain sexual development independently. Studies of SF-1 KO mice in adulthood have identified some sex differences in brain morphology and behavior traits in which genetic factors, acting independently or in concert with gonadal hormones, are likely contributors (11, 12).

The anteroventral periventricular nucleus (AVPV) in mice is sexually dimorphic for many traits such as volume, number of neurons (13) and in the size of chemically defined neuronal populations (e.g. number of cells expressing tyrosine hydroxylase or kisspeptin) (14, 15). All of the sex differences in the AVPV can be manipulated by changing gonadal hormonal milieu, showing the effect of gonadal hormones. In our study (11) we found that the number of neurons expressing neuronal nitric oxide synthase (nNOS) was higher in wild type (WT) males in comparison to WT females. A similar sex difference was found in SF-1 KO mice and since SF-1 KO mice are not exposed to gonadal hormones this suggests gonadal hormone independent sexual differentiation. We also studied expression of nNOS in the medial preoptic area (POA) where sex differences in brain morphology have been described previously in various species (rev. in (16)). As in the AVPV, SF-1 KO mice males had higher nNOS immunoreactive area than females, similar to the sex difference in WT mice (11). The stands in stark contrast to the grouping of cells containing immunoreactive calbindin in the same POA, for which the sex difference found was completely hormone-dependent (found in WT, but not KO) (11).

The ventromedial hypothalamus (VMH) is involved in regulation of various behaviors and endocrine processes (17). Structure of the VMH is sexually dimorphic and most sex differences have been found to be gonadal hormone dependent. Studies of VMH sex differences in SF-1 KO mice are complicated by alterations in its cyto-architecture (18). Nevertheless, similar sex differences in the number of calbindin D-28k immunopositive cells were found in WT and SF-1 KO mice suggesting gonadal hormone independent sexual differentiation (11).

Neuropeptide Y (NPY) is an orexigenic metabolic peptide, highly expressed in the arcuate nucleus, from where NPY neurons project to the paraventricular nucleus (rev. in (19)). NPY is also expressed in many other brain areas including the lateral septum where its function is not yet known. There are suggestions that the lateral septum may have important role(s) in social/ affiliative behaviors or in the regulation of food intake, at least in female rats (20). Interestingly, we found gonadal hormone independent sex difference in NPY expression in the lateral septum, similarly to previously described sexual dimorphism in arginine vasopressin expression in this area (7).

Conclusions

It is widely accepted that gonadal steroid hormones are the major factor influencing sex-dependent brain development. However recent studies have indicated that some genes may also have effects on shaping the brain acting independently or together with gonadal hormones in synergistic or antagonistic manners during development. Initial studies with two mouse models, SF-1 KO and FCG mice have already revealed several sex differences that are likely

dependent on sex chromosome gene complement, and future studies with these and other models will undoubtedly further reveal an interplay between sex hormones and genetic factors in shaping male or female brain structure and function.

Acknowledgements

This work was supported by NIH grant MH61376 (S.A.T. and G.M.), ICGEB grant CRP SLO 06/02, ARRS (Slovenian research agency) grants P4-0053 and J7-2093 (G.M.).

References

1. Phoenix CH, Goy RW, Gerall AA, et al. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Endocrinology 1959; 65: 369-82.

2. Dewing P, Chiang CW, Sinchak K, et al. Direct regulation of adult brain function by the male-specific factor SRY. Curr Biol 2006; 16: 415-20.

3. Xu J, Burgoyne PS, Arnold AP. Sex differences in sex chromosome gene expression in mouse brain. Hum Mol Genet 2002; 11: 1409-19.

4. Dewing P, Shi T, Horvath S, et al. Sexually dimorphic gene expression in mouse brain precedes gonadal differentiation. Brain Res Mol Brain Res 2003; 118: 82-90.

5. Carruth LL, Reisert I, Arnold AP. Sex chromosome genes directly affect brain sexual differentiation. Nat Neurosci 2002; 5: 933-4.

6. Arnold AP, Chen X. What does the "four core genotypes" mouse model tell us about sex differences in the brain and other tissues? Front Neuroendocrinol 2009; 30: 1-9.

7. De Vries GJ, Rissman EF, Simerly RB, et al. A model system for study of sex chromosome effects on sexually dimorphic neural and behavioral traits. J Neurosci 2002; 22: 9005-14.

8. Gatewood JD, Wills A, Shetty S, et al. Sex chromosome complement and gonadal sex influence aggressive and parental behaviors in mice. J Neurosci 2006; 26: 2335-42.

9. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 1994; 77: 481-90.

10. Majdic G, Young M, Gomez-Sanchez E, et al. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002; 143: 607-14.

11. Budefeld T, Grgurevic N, Tobet SA, et al. Sex differences in brain developing in the presence or absence of gonads. Dev Neurobiol 2008; 68: 981-95.

12. Grgurevic N, Budefeld T, Rissman EF, et al. Aggressive behaviors in adult SF-1 knockout mice that are not exposed to gonadal steroids during development. Behav Neurosci 2008; 122: 876-84.

13. Bleier R, Byne W, Siggelkow I. Cytoarchitectonic sexual dimorphisms of the medial preoptic and anterior hypothalamic areas in guinea pig, rat, hamster, and mouse. J Comp Neurol 1982; 212: 118-30.

14. Simerly RB, Swanson LW, Handa RJ, et al. Influence of perinatal androgen on the sexually dimorphic distribution of tyrosine hydroxylase-immunoreactive cells and fibers in the anteroventral periventricular nucleus of the rat. Neuroendocrinology 1985; 40: 501-10.

15. Clarkson J, Herbison AE. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology 2006; 147: 5817-25.

16. Tobet SA, Fox TO. Sex differences in neural morphology influenced hormonally throughout life. In: Gerall AA, Moltz H, Ward IL, eds. Handbook of Behavioral Neurobiology. New York: Plenum Press, 1992: 41-83.

17. McClellan KM, Parker KL, Tobet S. Development of the ventromedial nucleus of the hypothalamus. Front Neuroendocrinol 2006.

18. Ikeda Y, Luo X, Abbud R, et al. The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 1995; 9: 478-86.

19. Hillebrand JJ, de Wied D, Adan RA. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. Peptides 2002; 23: 2283-306.

20. Kovacs EG, Szalay F, Racz B, et al. Chronic fasting-induced changes of neuropeptide Y immunoreactivity in the lateral septum of intact and ovariectomized female rats. Brain Res 2007; 1153: 103-10.

OD SPOLNIH ŽLEZ NEODVISNE SPOLNE RAZLIKE V MOŽGANIH PRI MIŠIH BREZ GENA SF-1

T. Büdefeld, S. A. Tobet, G. Majdič

Povzetek: Spolne razlike v možganih so prisotne pri številnih živalskih vrstah vključno z ljudmi. Vplive spolnih hormonov na razvoj možganov so potrdili že pred 50 leti, medtem ko vplive genoma, natančneje spolnih kromosomov, ugotavljamo šele v zadnjem desetletju. V tem preglednem članku so opisani nekateri primeri vplivov genov na spolno diferenciacijo možganov, s poudarkom na miših brez gena *SF-1* (SF-1 knockout, SF-1 KO), ki predstavljajo poskusni model miši za proučevanje genetskih vplivov pri spolnem razvoju možganov.

Ključne besede: spolna diferenciacija možganov; spolni kromosomi; miši brez gena SF-1; predoptično področje in hipotalamus; živčna sintaza dušikovih oksidov; kalbindin D-28k, nevropeptid Y

Slov Vet Res 2010; 47 (4): 171-4
UDC 577.25:612.4:612.82

Review Article

TRANSCRIPTOME ANALYSIS OF BRAIN FROM STEROIDOGENIC FACTOR 1 KNOCKOUT MICE

Tanja Špenić[1]*, Tomaž Büdefeld[1], Gregor Majdič[1,2]

[1]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana; [2]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

*Corresponding author, E-mail: tanja.spanic@vf.uni-lj.si

Summary: The brain begins its life as neither male nor female and waits to be impacted by differences of genetic and hormonal actions that continue throughout the lifetime of an organism. Many differences have been described in the brain between sexes of a variety of species, ranging from amphibians and reptiles through birds and mammals, including humans. In our study the genomic-base array revealed six genes differentially expressed in brains from *SF-1* knockout males and females and their WT controls. All of these genes are sex chromosome-linked genes.

Key words: brain; sex difference; mouse; DNA microarray

Sex differences in body and brain

An origin of differences between sexes in mammals is in sex chromosomes with males having one X- and one Y-chromosome and females having two X-chromosomes. On the Y-chromosome there is a sex-determining region – *Sry* gene, which induces an undifferentiated gonads to form as testes rather than ovaries [1]. Testes then secrete hormones, testosterone and anti-Müllerian hormone, and the body develops in masculine fashion. If *Sry* is absent, gonads develop as ovaries, and the body forms in a feminine way [2]. In the last decade several studies in model organisms revealed that some sex differences in non-gonadal tissues are a consequence of differential effects of X-linked and Y-linked genes acting within non-gonadal cells [3; 4; 5; 6]. From these studies arises the question whether genes on sex chromosomes, which are present in different quantities in male and female genomes, might be expressed in the brain and might be partially responsible for a sex-specific model of development and/or function [7]. The non-recombinant region of the Y-chromosome (NRY) in males contains genes that are not present in females, and might act in the brain to cause masculine neural development. On the other hand, genes in the non-pseudoautosomal region of the X-chromosome (NPX), which are present in two copies in females but only in a single copy in males, could cause female-specific neuronal development [8]. Although one X chromosome is inactivated during development, a significant number of NPX genes escape inactivation, so the amount in the two sexes may not be equal [9].

The study of Xu *et al.* [8] with four core genotypes (FCG) mice (four genotypes are XY gonadal males (XYM), XX gonadal females (XXF), XX gonadal males (XXM) and XY gonadal females (XYF)) showed that some of the Y-linked genes (Usp9y, Ube1y, Smcy, Eif2s3y, Uty and Dby) do not require testicular secretion for sexual dimorphic expression in the brain as they were expressed in XYM and in XYF mice. In the same study they showed that six X-linked homologues (Usp9x, Ube1x, Smcx, Eif2s3x, Utx and Dbx) were also expressed in the brain, and in adulthood all of these transcripts were expressed at notably higher levels in female brains in comparison to male brains, regardless of their X-inactivation status. Several other studies revealed similar findings [10; 11].

SF-1 KO mice as a model for brain sexual differentiation

Steroidogenic factor 1 (SF-1, NR5A1) is a member of the nuclear receptor superfamily of transcription

Received: 4 October 2010
Accepted for publication: 12 November 2010

factors with important roles in the development and function of endocrine organs [12]. Mice lacking *SF-1* (*SF-1* knockout mice, *SF-1* KO) are born without adrenal glands and gonads; they have non-functional gonadotrope cells in the pituitary and ventromedial hypothalamus is not developed as a compact nucleus [13; 14]. Due to adrenal insufficiency they die shortly after birth. Early corticosteroid injections followed by adrenal transplantation can maintain *SF-1* KO mice into adulthood [15]. Because of early regression of gonads and adrenals, these mice are not exposed to endogenous sex steroids during development and are consequently an important model for studying hormone independent development of brain sex differentiation. Gonadal deficiency does not necessarily prevent exposure to sex steroids from other resource such as placenta, mother, or nearest siblings during fetal development, but these influence should be the same for all *SF-1* KO offspring [16].

For our studies, heterozygous *SF-1* KO mice were mated to obtain homozygous *SF-1* KO animals and wild type (WT) littermate controls. All newborn mice were treated with daily corticosteroid injections. After genotyping, a transplantation of adrenals to *SF-1* KO mice is performed on postnatal day 7 as described before [16]. All control WT mice are gonadectomized before puberty.

Current work

In our study we used the central part of an adult mouse brain, which involved the preoptic area, hypothalamus, amygdala, hippocampus and part of the cortex from WT control and SF-1 KO mice. After isolating the total RNA (mRNA), we performed a microarray experiment using mouse genomic-based array Affymetrix GeneChip®.

Data mining for differentially expressed genes revealed several genes that were statistically significantly different between sexes but not between genotypes. Interestingly, all candidate genes were sex chromosome linked, but validation of these results by quantitative RT PCR and/or in situ hybridization will be needed and is currently underway.

Conclusions

Since *SF-1* KO mice are not exposed to endogenous sex steroid hormones during development and after birth, changes in gene expression found in the microarray experiment must be sex hormone independent, as the same expression is present in WT and SF-1 KO animals.

Acknowledgements

We thank our colleagues at Faculty of Medicine in Ljubljana, especially Rok Košir, dr. Peter Juvan, and prof. dr. Damjana Rozman for helping us with microarry experiment and data analysis. Additionally, we thank these projects: ICGEB grant CRP SLO 06/02, ARRS (Slovenian research agency) grants P4-0053 and J7-2093 for necessary research funding.

Acknowledgements

This work was supported by ICGEB grant CRP SLO 06/02 and ARRS (Slovenian research agency) grants P4-0053 and J7-2093.

References

1. Goodfellow PN, Lovell-Badge R. SRY and sex determination in mammals. Annu Rev Genet 1993; 27: 71–92.

2. Wilson JD. The role of androgens in male gender role behavior. Endocr Rev 1999; 20(5): 726–37.

3. Renfree MB, Short RV. Sex determination in marsupials: evidence for a marsupial-eutherian dichotomy. Philos Trans R Soc Lond B Biol Sci 1988; 322(1208): 41–53.

4. Reisert I, Pilgrim C. Sexual differentiation of monoaminergic neurons–genetic or epigenetic? Trends Neurosci 1991; 14(10): 468–73.

5. Arnold AP. Genetically triggered sexual differentiation of brain and behavior. Horm Behav 1996; 30(4): 495–505.

6. Arnold AP, Burgoyne PS. Are XX and XY brain cells intrinsically different? Trends Endocrinol Metab 2004; 15(1): 6–11.

7. Arnold AP. Mouse models for evaluating sex chromosome effects that cause sex differences in non-gonadal tissues. J Neuroendocrinol 2009; 21(4): 377–86.

8. Xu J, Burgoyne PS, Arnold AP. Sex differences in sex chromosome gene expression in mouse brain. Hum Mol Genet 2002; 11(12): 1409–19.

9. Carrel L, Cottle AA, Goglin KC, Willard HF. A first-generation X-inactivation profile of the human X chromosome. Proc Natl Acad Sci U S A 1999; 96(25): 14440–4.

10. Yuge K, Ikeo K, Gojobori T. Evolutionary origin of sex-related genes in the mouse brain. Gene 2007; 406(1-2): 108–12.

11. Yang J, Zou Y, Zhu J. Identifying differentially expressed genes in human acute leukemia and mouse brain microarray datasets utilizing QTModel. Funct Integr Genomics 2009; 9(1): 59–66.

12. Lala DS, Rice DA, Parker KL. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. Mol Endocrinol 1992; 6(8): 1249–58.

13. Ikeda Y, Luo X, Abbud R, Nilson JH, Parker KL. The nuclear receptor steroidogenic factor 1 is essential for the formation of the ventromedial hypothalamic nucleus. Mol Endocrinol 1995; 9(4): 478–86.

14. Shinoda K, Lei H, Yoshii H, et al. Developmental defects of the ventromedial hypothalamic nucleus and pituitary gonadotroph in the Ftz-F1 disrupted mice. Dev Dyn 1995; 204(1): 22–9.

15. Majdič G, Young M, Gomez-Sanchez E, et al. Knockout mice lacking steroidogenic factor 1 are a novel genetic model of hypothalamic obesity. Endocrinology 2002; 143(2): 607–14.

16. Budefeld T, Grgurevič N, Tobet SA, Majdič G. Sex differences in brain developing in the presence or absence of gonads. Dev Neurobiol 2008; 68(7): 981–95.

ANALIZA TRANSKRIPTOMA MOŽGANOV MIŠI BREZ GENA SF-1

T. Španić, T. Büdefeld, G. Majdič

Povzetek: Možgani sprva niso niti moški niti ženski, temveč se diferencirajo pod vplivom različnih genetskih in hormonskih dejavnikov, ki so jim izpostavljeni skozi celotno življenje organizma. Razlike v možganih med spoloma so opisane že pri mnogih vrstah, od dvoživk in plazilcev preko ptic in sesalcev pa vse do človeka. V raziskavi s pomočjo DNK mikromrež smo odkrili šest genov, ki so različno izraženi v možganih samcev in samic miši brez gena *SF-1* in njihovimi kontrolnimi skupinami divjega tipa. Vseh šest genov se nahaja na spolnih kromosomih.

Ključne besede: možgani; spolne razlike; miš; DNK mikromreže

Slov Vet Res 2010; 47 (4): 175-80
UDC 577.25:615:616.8

Review Article

POTENTIAL APPLICATIONS OF DOPAMINE D1 AGONIST AND D2 ANTAGONIST LEK-8829

Marko Živin

Brain Research Laboratory, Institute of Pathophysiology, Medical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

E-mail: zivin@mf.uni-lj.si

Summary: Ergoline derivative 9,10-Didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829), possesses dopamine (DA) D1 agonistic and D2 antagonistic properties in the nigrostriatal and mesocorticolimbic DAergic pathways. These unique dual effects have suggested that LEK-8829 could effectively restore previously imbalanced functional linkage between D1 and D2 receptors under schizophrenic conditions in which, LEK-8829 could improve both the negative and positive symptoms of schizophrenia. As dopamine D1 receptor agonist, LEK-8829 may also be beneficial in relieving the motor symptoms of parkinsonism, alone, or when co-administered with antiparkinsonic dopamine D2 agonists, such as ergoline derivative bromocriptine. Moreover, antiparkinsonic potential of LEK-8829 may be particularly useful when the treatment of parkinsonism with D2 agonist drugs is complicated by psychosis. Antiparkinsonic properties of LEK-8829 also suggest a lower propensity of the drug for the induction of extrapyramidal syndrome in the treatment of schizophrenia. Furthermore, by blocking dopamine D2 receptors, LEK-8829 could block the incentive for drug-seeking and drug-craving while by stimulating dopamine D1 receptors it could mediate drug reward and gratification. This implies that LEK-8829 could also attenuate the relapse of psychostimulant drug-addiction, while not being addictive by itself.
We conclude that agents with LEK-8829-like dual actions toward dopamine receptors, may represent a new and potent drug class for the treatment parkinsonism, schizophrenia and drug-addiction.

Key words: LEK-8829; D1 agonist; D2 antagonist; antipsychotic; antiparkinsonic; antiaddictive

Introduction

The ergoline derivative, LEK-8829 (9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline), has been developed as a potential atypical antipsychotic drug with antagonistic actions at dopamine D2 and serotonin 5-HT2 and 5-HT1A receptors (1) in order to be more effective and/or produce fewer side effects than typical antypsichotics (neuroleptic) drugs, such as haloperidol. Although the blockade of dopamine D2 receptors is a prerequisite characteristic of clinically effective antipsychotic drugs, the blockade of dopamine D2 receptors within the basal ganglia often provokes unwanted extrpyramidal syndrome (EPS), characterized by parkinsonism, akathisia, catalepsy, and, after long-term treatment, tardive diskinesia (2). Fortunately, atypical antipsychotic drugs, such as clozapine, were developed that have a lower tendency for the induction of EPS. Clozapine is characterized by higher affinity ratio between serotonin 5-HT2 and dopamine D2 receptors (3). Low propensity for the induction of EPS is thought to depend on the ability of atypical antipsychotics to preferentialy inhibit mesolimbic dopaminergic (DA) system as opposed to neuroleptic drugs that effectively inhibit both mesolimbic and mesostriatal DA systems (4). However, the clinical usefulness of clozapine is hampered with the relatively high risk (1-2% of patients) of agranulocytosis or granulocytopenia (5). In addition to EPS, many currently used antipsychotics, including clozapine, exert other unwanted side effects, such as excessive sedation and signs of autonomic blockade with hypotension (6). Contrary to initial hope, atypical antipsychotics that have been developed so far are not very effective in the treatment of negative symptoms of schizophrenia.

Received: 15 October 2010
Accepted for publication: 12 November 2010

Ergoline derivatives may affect the central nervous system by an interaction with membrane receptors, including dopaminergic, adrenergic and serotonergic receptors. They can act either as agonists, partial agonists or antagonists at these receptors (7). Initially, the effects of ergoline LEK-8829 were compared to the effects of haloperidol and clozapine on various behavioral tests and in *in vitro* receptor binding studies. All compounds inhibited apomorphine-induced locomotor activity in rats, apomorphine-induced climbing behavior in mice and 5-hydroxytryptophan-induced head twitches in mice and induced catalepsy in rats and in mice. LEK-8829 and clozapine, but not haloperidol, showed a certain degree of mesolimbic selectivity, *i.e.*, they caused more potent inhibition of apomorphine-induced locomotion compared with the induction of catalepsy in rats. In the case of LEK-8829, nonspecific effects that presumably predict a side effect profile, such as potentiation of pentobarbital-induced anesthesia in mice (sedation), antagonism of oxotremorine-induced tremors in mice (anticholinergic activity), spontaneous locomotor activity in mice and norepinephrine-induced lethality in rats (sedation and hypotension), were relatively weak. The results of direct measurements of the influences of LEK-8829 on blood pressure showed that LEK-8829 was relatively weak hypotensive. It has been thus suggested that LEK-8829 might be an efficient antipsychotic with a reduced propensity to cause sedative, anticholinergic and hypotensive side effects. A certain degree of mesolimbic selectivity also pointed toward the possibility of a reduced propensity to cause EPS (8).

LEK-8829 has been shown to possess pure competitive antagonist activities at both 5-HT2 receptors and atpha-1 adrenoceptors in rabbit isolated aorta (7). *In vitro* radioligand binding studies revealed that LEK-8829 possess low affinity for rat striatal 3H-SCH23390-labeled dopamine D1 binding sites and high affinity for striatal 3H-spiperone-labeled D2 and cortical 3H-ketanserin labeled serotonin-2 (5-HT2) sites. The ratio of pKi values 5-HT1/D2 was 1.11 (closer to that of clozapine than haloperidol). Based on these experiments it has been concluded that LEK-8829 may be considered to have atypical antipsychotic potential (8).

Thereafter, the most important findings from behavioral, gene-expression and pharmacological studies in unilateral animal models of striatal dysfunction and cocaine self-administration have revealed that LEK-8829 is a dopamine D1 receptor agonist and D2 receptor antagonist drug.

D1 receptor agonist and D2 receptor antagonist properties of LEK-8829

Gene expression studies have shown that LEK-8829 is an up-regulator of the expression early response genes, such as c-*fos* and ANIA-4, and of the expression of genes for several neuropeptides expressed within the basal ganglia (preprotachykinin, neurotensin, and for opioides (preproenkephalin, dynorphin). In all experiments the regulation of the expression of neuropeptides by LEK-8829 was consistent with the effects of combined treatment with selective dopamine D2 receptor antagonists and D1 receptor agonists (9, 10). Interestingly, the above mentioned effects of LEK-8829 on gene expression within the basal ganglia were also consistent with the increased activity of adenylate cyclase within these subcortical nuclei. It is known, that within the striatum, both D2 antagonists and D1 agonists stimulate the activity of this enzyme.

However, this review focuses only on the findings from behavioral and pharmacological studies, and on speculation about the possible therapeutic roles of LEK-8829 in the treatment parkinsonism, schizophrenia, and the relapse of drug-addiction.

Potential antiparkinsonic effects on hypersensitive striatal dopamine receptors

Rats with unilateral dopaminergic denervation of the striatum, induced by the lesion of the median forebrain bundle with 6-hydroxydopamine (6-OHDA), are often used for *in vivo* screening of potential dopamine agonists or antagonists. The 6-OHDA model can be utilized for the evaluation of directly acting DA agonists, since these drugs induce contralateral (toward the intact side) turning behaviour (11). Furthermore, stimulation by partial 5-HTIA agonists can also induce contralateral turning (12).

On the DA lesioned side, denervational dopaminergic hypersensitivity develops. Upon stimulation with directly acting DA agonists, this results in dopaminergic striatal disbalance, since the stimulation of "hypersensitive" striatal dopamine receptors on the denervated side is more intensive compared to the stimulation of "normosensitive" striatal dopamine receptors on the intact side. In contrast, indirectly acting dopamine agonists, such as amphetamine, induce dopaminergic disbalance that results in ipsilateral turning (toward the lesioned

side), since these drugs could release dopamine only within striatum of the intact side. Since LEK-8829 was known to possess D2 antagonistic activity, we expected that LEK-8829 might inhibit the ipsilateral turning induced by amphetamine. Surprisingly, pretreatment with LEK-8829 *per se* induced long-lasting, dose-dependent contralateral turning behaviour, hinting its agonistic avctivity at dopamine receptors.

The receptor mechanism induced by LEK-8829 was then analyzed pharmacologically by the pretreatment of 6-OHDA-lesioned animals with antagonists of dopamine D1, D2 and 5HT1-A receptors, SCH23390, haloperidol and pindolol, respectively. It was found that only the specific D1 receptor antagonist SCH-23390 but not the D2 receptor antagonist haloperidol or 5-HT1A antagonist pindolol, dose-dependently inhibited the turning behaviour induced by LEK-8829. We concluded, therefore, that at least within DA hypersensitive striatum, LEK-8829 is having intrinsic activity at dopamine D1 receptors (9).

We also investigated the proposed D2 antagonistic activity of LEK-8829 at hypersensitive dopamine D2 receptors, by exploring the interaction of LEK-8829 with the dopamine D2 receptor agonist bromocriptine (2-bromo-alpha-ergokryptine). Treatment with either LEK-8829 or bromocriptine induced a vigorous contralateral turning response. Contralateral turning induced by the combined treatment was of similar intensity as the turning induced by single-drug treatments. These results may be explained by the known adaptations to long-term striatal dopamine depletion that result in the development of so called functional »uncoupling« of supersensitive dopamine receptors, where the locomotor stimulation induced by selective agonist of one type of dopamine receptors (e.g. D1) may not be blocked by the blockade of the other type of dopamine receptors (e.g. D2), in contrast to the inhibition of turning that occurs in models with intact, "functionally coupled" dopamine receptors. Accordingly, in our experiment, the pretreatment with selective D1 antagonist SCH-23390 did not have a significant effect on bromocriptine-induced turning, but significantly decreased the turning observed after the combined LEK-8829/bromocriptine treatment (13). We also found that LEK-8829 inhibited contralateral turning induced by D2 agonist quinpirole, but again, only if the rats were co-treated with SCH-23390 (10). We reasoned, that in the 6-OHDA model, the contralateral turning mediated by tLEK-8829, occurs due to intrinsic activity of LEK-8829 on dopamine D1 receptors, while the contralateral turning induced by bromocriptine may be inhibited by concomitant D2 antagonistic activity of LEK-8829. As D1 agonist, LEK-8829 thus by itself has an antiparkinsonic potential that may be particularly useful in situations when the treatment of parkinsonism with D2 agonists, such as with bromocriptine, is complicated by psychosis provoked by over-stimulation of dopamine D2 receptors.

Potential antypschotic effects on normosensitive striatal dopamine receptors

In contrast to parkinsonism, denervational dopaminergic hypersensitivity does not seem to be the underlying mechanism of derranged dopaminergic activity in schizophrenia. Instead, the dopaminergic concept of schizophrenia pathogenesis is based on regional imbalance of brain DA function that arises from dysfunction of D1 receptors in the medial prefrontal cortex (mPFC) and hyperactivity of D2 receptors in ventral tegmental area (VTA) and nucleus accumbens (NAc) (14). The hypothesis of the above regional receptor disbalance in schizophrenia has resulted in the prediction that LEK-8829 may serve also as a potential candidate for the treatment of the negative symptoms schizophrenia.

Rats with unilateral striatal lesions with ibotenic acid (IA) may be used for analysis of pharmacological effects of the drug on presumably normosensitive striatal dopamine receptors. In contrast to unilateral model of parkinsonism, in IA model the rats circle ipsilaterally (toward the lesioned side) when challenged either with directly or indirectly acting DA agonists. Unexpectedly, LEK-8829 induced a dose-dependent contralateral turning also in IA model. Like in 6-OHDA model, LEK-8829-induced contralateral turning was blocked by D1 receptor antagonist SCH-23390. We assumed that contralateral turning in unilateral IA model may be a consequence of simultaneous blockade of dopamine D2 and stimulation of dopamine D1 receptors. Accordingly, we found that the combined treatment with D1 receptor agonist SKF-82958 and D2 antagonist haloperidol also resulted in contralateral turning of IA rats. In control rats, the treatment with SKF-82958 induced ipsilateral turning, whereas the treatment with haloperidol induced contralateral posture. When the rats were treated first with LEK-8829 followed with bromocriptine, the rats changed the direction of turning from contralateral to the ipsilateral side. This result was interpreted as the

consequence of the competition of bromocriptine with LEK-8829 at normosensitive dopamine D2 receptors. We reasoned that depending on the concentration ratio bromocriptine/LEK-8829 at dopamine receptors, bromocriptine could displace LEK-8829 from dopamine D2 receptors (and vice versa). If stimulatory activity of bromocriptine prevails, this results in ipsilateral turning due to co-stimulation of dopamine D2 and dopamine D1 receptors, by bromocriptine and LEK-8829, respectively (15).

Microinjection experiments with LEK-8829 in mPFC, VTA and NAc shall be performed in the future, to determine its D1 stimulation/D2 inhibition effects within the brain regions known to be involved in positive and negative symptoms of schizophrenia.

Potential antiaddictive effects on dopamine receptors within the reward system

In clinical studies, dopamine D1 agonists and D2 antagonists have been used with limited success for cocaine addiction treatment. The main disadvantage of selective D1 agonists as potential treatment medications is their reinforcing and thus abuse potential and selective D2 antagonists have an unfavorable profile of side-effects, since they commonly induce severe EPS.

Self-administration studies show that selective dopamine D1 or D2 receptor agonists have reinforcing properties and can mimic the discriminative stimulus produced by cocaine and stimulate locomotor activity. In contrast to their synergistic responses in most physiological and behavioral actions, dopamine D1 and D2 receptors seem to have opposing effects on relapse to cocaine-seeking behaviour (16). Some studies have shown that while systemic injection of selective D2 agonists potentiates the ability of cocaine to induce cocaine-seeking and that D2 agonists themselves induce cocaine-seeking behaviour, selective D1 agonists attenuate the ability of cocaine to induce cocaine-seeking behaviour and suppress the initiation of cocaine self-administration. These findings suggest that D2-like dopamine receptors could mediate the incentive for drug seeking and promote drug craving while D1-like dopamine receptors could mediate drug reward and gratification (17). In this regard it is noteworthy that drug reward and gratification may be conveyed by synergistic stimulation of the expression of endogenous opioides within the reward system by the above mentioned dual pharmacological profile of LEK-8829 at dopamine D1 and D2 receptors.

The extinction and reinstatement paradigm of animal drug self-administration is considered as a model of human drug-craving and relapse. We have used the model of *i.v.* self-administration of cocaine by rats to test the effects of LEK-8829 on reinstatement of extinguished cocaine-seeking and on cocaine self-administration. We speculated that by concomitant stimulation of D1 receptors and inhibition of D2 receptors, LEK-8829 might attenuate reinstatement of cocaine-seeking induced by cocaine injection and serve at the same time as maintenance and as antagonist drug. In view of its D1 agonistic effects, LEK-8829 was also tested for its reinforcing properties. We have found that the pretreatment with systemic injections of LEK-8829 attenuated reinstatement of cocaine seeking induced by cocaine priming injections and diminished cocaine intake in cocaine self-administration sessions. LEK-8829 itself did not induce reinstatement of cocaine- seeking and did not maintain intravenous self-administration (18). These findings indicate that LEK-8829 is a candidate medication for the treatment of cocaine craving in cocaine addiction. As mentioned above, LEK-8829 was also found to increase the synthesis and release of endogenous striatal opioid peptides, an action that may contribute to its anti-addictive potential.

Conclusion Remark

Although many questions regarding the beneficial mechanisms of LEK-8829 in parkinsonism, schizophrenia and drug-addiction remain to be addressed, it appears that agents with dual actions toward DA receptors may represent a new and potent drug class for the treatment of these disorders. LEK-8829 may be particularly useful whenever the treatment of parkinsonism with D2 agonist drugs, is complicated by psychosis. Antiparkinsonic properties of LEK-8829 also suggest a lower propensity of this drug for the induction of EPS in the treatment of positive symptoms of schizophrenia. Furthermore, by blocking dopamine D2 receptors, LEK-8829 could block the incentive for drug-seeking and drug-craving while by stimulating dopamine D1 receptors it could mediate drug reward and gratification. This implies that LEK-8829 could also attenuate the relapse of psychostimulant drug-addiction, while not being addictive by itself.

Acknowledgements

This work was supported by Slovenian Research Agency, grants No. 7885-0381, J-3 8722, P3 521 381, P3-0171, grant by LEK, Pharmaceutical and Chemical Co., Ljubljana, Slovenia and by STSM grant from COSTB10.

References

1. Krisch I, Rucman R, Lavric A, Ocvirk M, Bole-Vunduk B. A new ergoline derivative, LEK-8829, as a potential new antipsychotic drug. CNS Drug Rev 1996; 2: 294–307.

2. Deniker P. The neuroleptics: a historical survey. Acta Psychiatr Scand Suppl 1990; 358:83–7.

3. Meltzer HY, Matsubara S, Lee JC. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin2 pKi values. J Pharmacol Exp Ther 1989; 251:238–46.

4. Scatton B, Sanger DJ. Pharmacological and molecular targets in the search for novel antipsychotics. Behav Pharmacol 2000; 11:243–56.

5. Meltzer HY, Gudelsky GA. Dopaminergic and serotonergic effects of clozapine. Arzneim Forsch 1992; 42: 268–72.

6. Schwartz JT, Brotman AW. A clinical guide to antipsychotic drugs. Drugs 1992; 44: 981–92.

7. Krisch I, Budihna MV, Rucman R. Structure-activity study of some newly synthesized ergoline derivatives on 5-HT2 receptors and alpha adrenoceptors in rabbit isolated aorta. Pharmacology (Basel)1992; 45: 195–208.

8. Krisch I, Bole-Vunduk B, Pepelnak M, et al. Pharmacological studies with two new ergoline derivatives, the potential antipsychotics LEK-8829 and LEK-8841. J Pharmacol Exp Ther 1994; 271:343–52.

9. Zivin M, Sprah L, Sket D. The D1 receptor-mediated effects of the ergoline derivative LEK-8829 in rats with unilateral 6-hydroxydopamine lesions. Br J Pharmacol 1996; 119:1187–96.

10. Glavan G, Sket D, Zivin M. Modulation of neuroleptic activity of 9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829) by D1 intrinsicactivity in hemi-parkinsonian rats. Mol Pharmacol 2002; 61:360–8.

11. Sonsalla PK, Manzino L, Heikkila RE. Interactions of D1 and D2 dopamine receptors on the ipsilateral vs. contralateral side in rats with unilateral lesions of the dopaminergic nigrostriatal pathway. J Pharmacol Exp Ther 1988; 247:180–5.

12. Gerber R, Altar CA, Liebman JM. Rotational behavior induced by 8-hydroxy-DPAT, a putative 5HT-1A agonist, in 6-hydroxydopamine-lesioned rats. Psychopharmacology (Berl) 1988; 94:178–82.

13. Zivin M, Sprah L, Sket D. Antiparkinsonian potential of interaction of LEK-8829 with bromocriptine. Eur J Pharmacol 1998; 349:151–7.

14. Goldman-Rakic PS, Muly EC 3rd, Williams GV. D1 receptors in prefrontal cells and circuits. Brain Res Brain Res Rev 2000; 31: 295–301.

15. Sprah L, Zivin M, Sket D. Ergoline derivative LEK-8829-induced turning behavior in rats with unilateral striatal ibotenic acid lesions: interaction with bromocriptine. J Pharmacol Exp Ther 1999; 288: 1093–100.

16. Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science 1996; 271:1586–9.

17. Self DW, Nestler EJ. Relapse to drug-seeking: neural and molecular mechanisms. Drug Alcohol Depend 1998; 51: 49–60.

18. The dopamine D1 receptor agonist and D2 receptor antagonist LEK-8829 attenuates reinstatement of cocaine-seeking in rats. Naunyn Schmiedbergs Arch Pharmacol 2004; 369: 576–82.

POTENTCIALNA UPORABNOST LEK-8829, AGONISTA DOPAMINSKIH RECEPTORJEV D1, IN ANTAGONISTA DOPAMINSKIH RECEPTORJEV D2

M. Živin

Povzetek: Ergolinski derivat 9,10-didehydro-N-methyl-(2-propynyl)-6-methyl-8-aminomethylergoline bimaleinate (LEK-8829) je agonist dopaminskih receptorjev D1 in antagonist dopaminskih receptorjev D2 v nigrostriatnem in mezolimbično-kortikalnem dopaminergičnem sistemu. Ta edinstveni dvojni receptorski učinek LEK-8829 nakazuje možnost poprave posledic funkcionalnega razklopa dopaminskih receptorjev D1 in D2 pri bolnikih s shizofrenijo, pri čemer bi zato LEK-8829 lahko ublažil tako pozitivne kot negativne simptome te duševne bolezni. Samostojno ali skupaj z ergolinskim derivatom bromokriptinom, antiparkinsonikom z agonističnim delovanjem na dopaminskih receptorjih D2 bi LEK-8829 s spodbujanjem dopaminskih receptorjev D1 lahko ublažil motorične simptome parkinsonizma, z zaviranjem dopaminskih receptorjev D2 pa bi hkrati zmanjševal nevarnost za nastanek psihoze, ki je možen zaplet zdravljenja parkinsonizma z agonisti dopaminskih receptorjev D2. Antiparkinsonski učinki LEK-8829 obetajo tudi manjšo nagnjenost LEK-8829 za povzročanje ekstrapiramidnega sindroma pri zdravljenju shizofrenije z LEK-8829. Še več, z zaviranjem dopaminskih receptorjev D2, bi LEK-8829 pri osebah zasvojenih s psihomotoričnimi stimulansi, morda zmanjšal intenzivnost apetitivnega vedenja (hlepenja) povezanega z iskanjem droge, s spodbujanjem dopaminskih receptorjev D1 pa bi, s posnemanjem nagrajevalnih in hedonističnih učinkov droge, ublažil posledice umanjkanja teh učinkov med abstinenco. Tako bi LEK-8829 lahko preprečeval recidiv zasvojenosti, pri čemer pa sam ne bi imel zasvojevalnega učinka.
Sklepamo, da bi snovi z dvojnim učinkom na dopaminskih receptorjih, tako kot LEK-8829, lahko predstavljale novo vrsto učinkovitih zdravil za zdravljenje parkinsonizma, shizofrenije in zasvojenosti z drogami.

Ključne besede: LEK-8829; D1 agonist; D2 antagonist; antipsihotik; antiparkinsonik; antiaditiv

Slov Vet Res 2010; 47 (4): 181
UDC 575.112:577:576

MATHEMATICAL MODELING OF BIOLOGICAL EVENTS AND CELL-CELL COMMUNICATION

Steve Benoit, Vakhtang Putkaradze

Department of Mathematics; Colorado State University, Fort Collins, Colorado, USA

The modeling of cellular migration and intracellular communication at the scale of small to medium sized collections of cells is challenging due to the complexity of the systems involved and the diversity of their behavior (1). Several attempts at constructing models have been made (e.g., 2, 3), and fall into two categories. Top-down models begin with phenomenology and attempt to model behavior using either deterministic or stochastic methods, while bottom-up models begin with molecular dynamics or measurable physical properties of cell components and attempt to derive larger-scale behavior. To date, the top-down models have failed to reproduce observed cell behaviors during migration and tissue formation, and bottom-up models cannot simulate a large enough collection of cells for sufficient time to produce testable predictions. A new class of model is needed that can predict cell behavior at a scale between the limits of current top-down and bottom-up models.

The analyses begin with examples of cell motion measured in live tissue (4, 5), and pursue a phenomenological analysis to demonstrate the challenges of constructing a model of this system. Cell trajectories are extracted, mean squared displacements of cells over time are measured, and cells are classified according to the exponent in a best-fit diffusive model of this data (subdiffusive, diffusive, or superdiffusive) as well as speed and direction of motion, then these measures are correlated with tissue domains in the sample. Results show distinctly different cell behavior over the visualized tissue region.

Finally, we present highlights of our models of cell components that attempt to fill this modeling middle ground, including a cell membrane model based on discs that exhibit Lennard-Jones interactions in the transverse plane and elastic membrane forces in the axial direction, a cytoskeleton model consisting of Lennard-Jones spheres that change size or divide based on a regional polymerization/depolymerization bias created by diffusion of signaling chemical, and an extension of the membrane model to organelles with cells. These models, in combination, demonstrate chemotactic behavior with both attractive and repulsive signals, and take on expected membrane deformations and cell shapes in aggregations.

References

1. Tomita M. Whole-cell simulation: a grand challenge of the 21st century. Trends Biotechnol 2001; 19(6): 205–10.

2. Cickovski TM, Huang C, Chaturvedi R, et al. A framework for three-dimensional simulation of morphogenesis. IEEE/ACM Trans Comput Biol Bioinform 2005; 2(4):273–88.

3. Takahashi K, Kaizu K, Hu B, Tomita, M. 2004 A multi-algorithm, multi-timescale method for cell simulation. Bioinformatics. 20(4):538–546.

4. McClellan KM, Calver AR, Tobet SA. $GABA_B$ receptors role in cell migration and positioning within the ventromedial nucleus of the hypothalamus. Neuroscience 2008; 151(4):1119–31.

5. Tobet SA, Walker HJ, Seney ML, Yu KW. Viewing cell movements in the developing neuroendocrine brain. Integr Comp Biol 2003; 43(6):794–801.

Slov Vet Res 2010; 47 (4): 182
UDC 577:616.4

SUSCEPTIBILITY OF THE VASCULARIZATION IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO ALTERED GABA RECEPTOR SIGNALING, ENDOGENOUS SEX HORMONES, AND PRENATAL STRESS

Krystle A. Frahm[1], Tomaž Büdefeld[2], Gregor Majdič[2,3], Stuart A. Tobet[1]

[1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523-1680, USA; [2]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana; [3]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

The paraventricular nucleus (PVN) of the hypothalamus plays important roles in regulating sympathetic vasomotor tone, food intake, stress responses and cardiovascular function (1). The PVN also contains a denser matrix of blood vessels than the surrounding brain regions that develops postnatally in rats (2) and mice (3). A series of studies are being conducted to determine factors that are important for the development of this unique vascularization. Antisera directed against platelet endothelial cell adhesion molecule, which is present on endothelial cells that line blood vessels, were used to visualize large and small diameter blood vessels by immunohistochemistry. $GABA_B$ receptors play a role in PVN development during fetal life (4) and mice lacking the R1 subunit of the $GABA_B$ receptor were examined to see if this influence extends to the postnatal vascularization. Vascular branching was taken as an index of vascularization in a region of interest inside the PVN. Results showed $GABA_B$ receptor knockout mice had a significant decrease in vascular branching than wild type control mice on postnatal days 19 and 20 (5). There was a trend for females to have more branch points than males in $GABA_B$R1 subunit knockout and control, indicating that sex hormones may also play a role during development. Since endothelial cells contain estrogen receptor β (ERβ), this suggests the potential for circulating sex hormones to alter the density in vascularization in the PVN. To test this hypothesis, steroidogenic factor 1 knockout (SF-1 KO) mice are being used. SF-1 is a key regulator of gonadal and adrenal development (6). SF-1 KO mice are born without gonads and adrenal glands and are not exposed to endogenous gonadal sex steroid hormones. Therefore, it is hypothesized that male SF-1 KO mice will have more branch points compared to wild type. In addition, the synthetic glucocorticoid dexamethasone has been shown to increase the number of endothelial cells in vitro (7), which suggests the potential to increase angiogenesis in vivo. Dexamethasone is administered prenatally for proper lung development in humans, but the extent this plays on the developing vascularization in brain is unknown. To being to test this role in a preliminary experiment, dexamethasone was injected into pregnant heterozygous SF-1 KO mice from embryonic days 11 to 17. Results will determine if excess levels of glucocorticoid stimulation, alone or in combination with the lack of endogenous sex steroids during postnatal development, will alter the vascularization in the PVN. Changes in vascular branching may alter the ability of the PVN to properly receive signals and respond appropriately.

References

1. Swanson LW, Sawchenko PE. Hypothalamic integration rganization of the paraventricular and supraoptic nuclei. Annu Rev Neurcosci 1983; 6: 269–324.

2. Menendez A, Alvarez-Uria M. The development of vascularization in the postnatal rat paraventricular nucleus: a morphometric analysis. J Hirnforsch 1987; 28(3): 325–9.

3. Schow MJ, Knoll JG, Frahm KA, Zhang Q, Tobet SA. Abstracts of the 40th Annual Meeting of the Society for Neuroscience. San Diego, 2010.

4. McClellan KM, Stratton MS, Tobet SA. Roles for gamma-aminobutyric acid in the development of the paraventricular nucleus of the hypothalamus. J Comp Neurol 2010; 518(14): 2710–28.

5. Frahm KA, Schow MJ, Tobet SA. Abstracts of the 40th Annual Meeting of the Society for Neuroscience. San Diego, 2010.

6. Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 1994; 77(4): 481–90.

7. Maca RD, Fry GL, Hoak JC. The effects of glucocorticoids on cultured human endothelial cells. Br J Haematol 1978; 38(4): 501–9.

Slov Vet Res 2010; 47 (4): 183
UDK 576.32/.36

MODELS OF SIMPLE CELL AGGREGATES

A. Hočevar[1], S. El Shawish[1], P. Ziherl[1,2]

[1]Jožef Stefan Institute, Jamova 39, [2]Faculty of Mathematics and Physics, University of Ljubljana, Jadranska 19, 1000 Ljubljana, Slovenia

En-face cross-section of simple biological tissues such as planar epithelia resembles polygons that tile the plane. We show that the structure of such tissues can be explained by an equilibrium model where energy degenerate polygons in an entropy-maximizing tiling are described by a single geometric parameter reduced area a measuring their roundedness [1]. Tilings found numerically are in good agreement with experimental patterns observed in Drosophila, Hydra, and Xenopus. The geometric constraint demanding that polygonal cells must tile the plane without gaps or overlaps prevails over other mechanisms that mold a tiling, suggesting that there may be a universal mechanism that controls its structure. To explore this idea, we extend our analysis to other biological tissues as well as geological formations, supermagnetic froths, soap foams, and patterns seen in tabletop experiments. We characterize the tilings by their distributions of polygon reduced area and show that the structure of a random two-dimensional cellular partition, encoded by the frequencies of polygon classes, can be parametrized by its median reduced area alone.

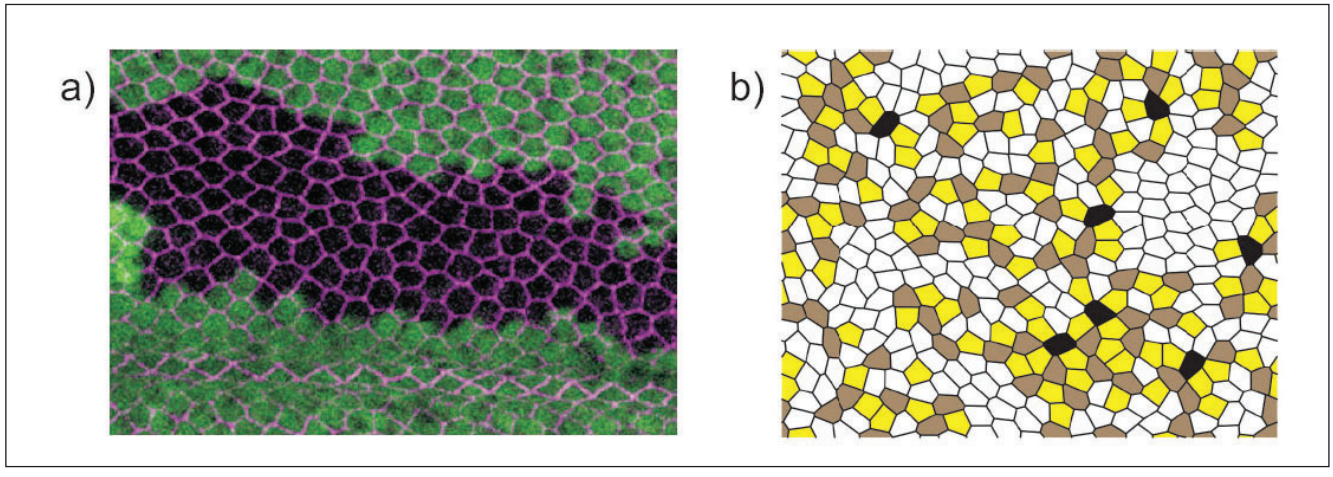

Figure 1: Panel a) illustrates a Drosophila wing epithelium [2]. A simulated tiling at reduced area a = 0.82 is shown in panel b). Different colors represent different polygon classes

References

1. Hocevar A, Ziherl P. Degenerate polygonal tilings in simple animal tissues. Phys Rev E Stat Nonlin Soft Matter Phys 2009; 80(1): 011904.

2. O'Keefe DD, Gonzalez-Niño E, Burnett M, et al. Rap1 maintains adhesion between cells to affect Egfr signaling and planar cell polarity in Drosophila. Dev Biol 2009; 333: 143–60.

Slov Vet Res 2010; 47 (4): 184-5
UDC 57.08:577.25:612.6.05:611.815

FORMING FUNCTIONAL CELL GROUPS IN THE DEVELOPING BRAIN

Matt Stratton[1], Tomaž Büdefeld[2], Gregor Majdič[2,3], Stuart Tobet[1]

[1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523-1680, USA; [2]Center for Animal Genomics, Veterinary faculty, University of Ljubljana, Gerbičeva 60, 1000 Ljubljana; [3]Institute of Physiology, Medical School, University of Maribor, Slomškov trg 15, 2000 Maribor, Slovenia

The complexity of the adult brain originates from a simple developmental structure called the neural tube. There are a number of cellular developmental processes that occur in an orchestrated fashion to mediate the transition from neural tube to adult brain. These include processes such as cell division, migration, and specification, process outgrowth, and path finding, in addition to programmed or signal induced cell death (often apoptosis). These processes are usually dependent on a combination of internal programming and external cues. While the formation of layered structures such as seen in the cortex has been well studied (1), the formation of nuclear cell groups in the hypothalamus is less understood. Hypothalamic nuclear groups are heterogeneous populations of cells that regulate the autonomic nervous system, motivated behavior and endocrine balance. Neurons in the paraventricular nucleus (PVN) of the hypothalamus, for example, control body temperature and metabolism via thyrotropin releasing-hormone containing cells, blood pressure via vasopressin containing cells, stress responses via corticotropin releasing hormone and vasopressin containing cells, reproductive function via oxytocin containing cells. The PVN also integrates information from other brain regions and samples hormone and emotional status to determine appropriate output/activity of its various cell types (2). From a developmental perspective the molecular specificity needed to direct cells to the correct location, initiate correct gene expression and connect to the appropriate circuits presents a difficult problem to solve. It has been hypothesized that small interferences (genetic or environmental) to this process cause changes in cytoarchitecture that impact adult physiology and contribute to pathology (3). A limited number of signaling pathways thought important for the development of hypothalamic cell groupings have been identified. $GABA_B$ receptor signaling may play important roles in formation of the PVN. For one, components of the pathway are expressed in a spatiotemporal pattern suggesting it could be important for PVN development (4). Secondly, dysregulation of $GABA_B$ signaling pathways or genetic mutation of components in the pathway have been associated with disorders resulting from altered PVN function (5, 6). Previous studies have shown that when the $GABA_B$ signaling pathway is disrupted, cytoarchitecture and peptide expression in the PVN is altered (4, 7). In this pilot study mice were embryonically exposed to the $GABA_B$ receptor antagonist 2-hydroxy-saclofen and adult anxiety like behaviors and depressive like behaviors were assayed using the elevated plus maze (8) and forced swim (9) test, respectively. Preliminary data suggests that embryonic saclofen exposure caused increased depressive like behaviors as indicated by increased floating time in the forced swim test. We suggest that developmental disruption of $GABA_B$ receptor signaling can cause changes in cyto-architecture that may alter adult physiology and behavior.

References

1. Sansom SN, Livesey FJ. Gradients in the brain: the control of the development of form and function in the cerebral cortex. Cold Spring Harb Perspect Biol 2009; 1(2): a002519.

2. Ferguson AV, Latchford KJ, Samson WK. The paraventricular nucleus of the hypothalamus - a potential target for integrative treatment of autonomic dysfunction. Expert Opin Ther Targets 2008; 12(6): 717–27.

3. Tobet SA, Henderson RG, Whiting PJ, Sieghart W. Special relationship of gamma-aminobutyric acid to the ventromedial nucleus of the hypothalamus during embryonic development. J Comp Neurol 1999; 405(1): 88–98.

4. McClellan KM, Stratton MS, Tobet SA. Roles for gamma-aminobutyric acid in the development of the paraventricular nucleus of the hypothalamus. J Comp Neurol 2010; 518(14): 2710–28.

5. Cryan JF, Kaupmann K. Don't worry 'B' happy!: a role for GABA(B) receptors in anxiety and depression. Trends Pharmacol Sci 2005; 26(1): 36–43.

6. Frankowska M, Filip M, Przegalinski E. Effects of GABAB receptor ligands in animal tests of depression and anxiety. Pharmacol Rep 2007; 59(6): 645–55.

7. Stratton MS, Schow M, Dennison C, Knoll JG, Tobet SA. GABA regulates the levels of immunoreactive corticotropin releasing hormone in the paraventricular nucleus of newborn female but not male mice. In: 92nd Annual Meeting of the Endocrine Society. San Diego, CA, 2010: P1-200.

8. Dawson GR, Tricklebank MD. Use of the elevated plus maze in the search for novel anxiolytic agents. Trends Pharmacol Sci 1995; 16(2): 33–6.

9. Pollak DD, Rey CE, Monje FJ. Rodent models in depression research: classical strategies and new directions. Ann Med 2010; 42(4): 252–64.

AUTHOR INDEX VOLUME 47, 2010

EN KLIC DO INFORMACIJSKO IN KOMUNIKACIJSKO OPREMLJENE PISARNE!

100% ZAŠČITA PRED IZGUBO PODATKOV

+

+

MICROSOFT OFFICE

INTERNET

+

ZAŠČITA

+

SERVIS

TELEFON
01 • 47 46 556

www.flipit.si

Nakup računalnika ima svojo ceno. Programska oprema in zaščita prav tako. Dokler deluje.
Naša vrednost se meri v 100% zanesljivosti. 100% zaščiti. 365 dni na leto.

flipit

INSTRUCTIONS FOR AUTHORS

Slovenian Veterinary Research contains original articles which have not been published or considered for publication elsewhere. All statements in the articles are the responsibility of the authors. The editorial policy is to publish original research papers, review articles, case reports and abstracts of theses, as well as other items such as critical reviews of articles published in Slov Vet Res, shorter scientific contributions, letters to the editor, etc. Authors should send their contributions to the editorial board's address. All articles are subjected to both editorial review and review by an independent referees selected by the editorial board. The editorial board reserves the right to translate titles, summaries and keywords that have not been translated into Slovene by the authors.

Contributions should be written in English and should not exceed 12 pages (27 lines per page, approx. 75 characters per line). They should be submitted electronically (preferably to E-mail address, slovetres@vf.uni-lj.si), written in any word processor for Windows. Authors are requested to provide names of three potential reviewers. The text should be double spaced and the lines should be numbered on the left-hand side. The margin on the left-hand side of the page should be 4 cm.

The front page of a manuscript should start with the title, followed by the name and surname of the author(s). If there is more than one author, their names should be separated by commas. The next line ('Addresses of authors:') should contain the authors' full names and addresses (institution, street and number, postcode and place) after the colon. All the given data should be separated by commas. The name, address and E-mail and/or phone number of the corresponding author should be written in the next line.

The Summary of 200-300 words should follow on the next page.

Under 'Keywords:' (after the colon), keywords should be given. Individual words or word combinations should be separated by semicolons. Scientific papers and papers which present the author's research and findings should also include the following obligatory headings assigned by the author to appropriate parts of the text: Introduction, Materials and methods, Results, Discussion, and References. Review articles should consist of an introduction, sections logically titled according to the content, and references. Information on fund-providers and other matters important for the paper (e.g. technical assistance) should be supplied under 'Acknowledgements', which should be placed before the references. Figure legends should follow the references.

Tables, graphs and diagrams should be logically incorporated in the text file. Original photographs or drawings should be sent as separate files in bmp, jpg or tif format. They should be referred to by type and using Arabic numerals (e.g. Table 1:, Figure 1:, etc.). The colon should be followed by the text or title. All references cited in the text should appear in the References. They should be numbered in the text in the order in which they appear, marked with Arabic numerals placed in parenthesis. The first reference in the text should determine the number and order of the respective source in the References. If the author refers again to a source which has already been used in the text, he should cite the number the source had when it was referred to for the first time. Only works which have been published or are available to the public in any other way may be referred to. Unpublished data, unpublished lectures, personal communications and similar should be mentioned in the references or footnotes at the end of the page on which they appear. Sources in the References should be listed in the order in which they appear in the text. If the source referred to was written by six authors or less, all of them should be cited; in the case of seven or more authors, only the first three should be cited, followed by 'et al.'.

Any errata should be submitted to the editor-in-chief in good time after publication so that they may be published in the next issue.

Examples of references

Book: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Chapterorar ticle in a book: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Article in a journal or newspaper: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Article in proceedings of a meeting or symposium: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

NAVODILA AVTORJEM

Slovenski veterinarski zbornik (Slovenian Veterinary Research) objavlja izvirne prispevke, ki še niso bili objavljeni oz. poslani v objavo drugam. Za vse navedbe v prispevkih so odgovorni avtorji. Uredniška politika obsega publiciranje znanstvenih člankov, preglednih znanstvenih člankov, strokovnih člankov, povzetkov disertacij in drugih prispevkov, kot so kritične presoje o vsebini razprav, objavljenih v zborniku, kratke znanstvene prispevke, pisma uredniku in drugo. Avtorji pošljejo prispevke na naslov uredništva. Glavni urednik pregleda vse prispevke. Za vse članke je obvezna strokovna recenzija, za katero poskrbi uredništvo.

Prispevki naj bodo napisani v angleškem jeziku, z naslovom, povzetkom in ključnimi besedami tudi v slovenščini. Obsegajo naj največ 12 strani, kar pomeni 27 vrstic na stran s približno 75 znaki v vrstici. Prispevki naj bodo poslani v elektronski obliki v katerem koli urejevalniku besedil za okensko okolje. Zaželjena je uporaba elektronske pošte (slovetres@vf.uni-lj.si) in avtorji naj predlagajo tri možne recenzente. Besedilo naj ima dvojni razmik med vrsticami, pri čemer naj bodo vrstice na levi strani oštevilčene. Besedilo naj bo na levi strani od roba oddaljeno 4 cm.

Naslovna stran prispevkov se začne z naslovom, sledi ime in priimek avtorja. Kadar je avtorjev več, jih ločimo z vejicami. V naslednjih vrsticah je v rubriki Addresses of authors: za dvopičjem treba navesti polno ime in priimek ter naslov(e) avtorja(ev), tj. ustanovo, ulico s hišno številko, pošto in kraj. Vse navedene podatke ločujejo vejice. Sledi vrstica, kjer je treba navesti ime ter elektronski (E-mail:) in poštni naslov ter telefonsko številko (Phone:) odgovornega avtorja.

Sledi besedilo povzetka Summary v obsegu 200 do 300 besed. V naslednji rubriki Key words: se za dvopičjem navedejo ključne besede. Posamezne besede ali sklopi besed morajo biti ločeni s podpičjem.

Znanstveni članki in tisti, ki so prikaz lastnih raziskav in dognanj, morajo vsebovati še naslednje obvezne rubrike, s katerimi avtor sam naslovi ustrezne dele besedila v prispevku: Introduction, Material and methods, Results, Discussion in References. Pregledni članki naj vsebujejo uvod, poglavja, ki so glede na vsebino smiselno naslovljena, in literaturo. Podatke o financerjih ali drugih zadevah, pomembnih za prispevek, npr. o tehnični pomoči, avtorji navedejo v rubriki Acknowledgements, ki se uvrsti pred rubriko References. Za rubriko References sledijo spremna besedila k slikam.

Priloge, kot so tabele, grafikoni in diagrami naj bodo smiselno vključene v besedilo. Slikovni material naj bo poslan posebej v obliki bmp, jpg, ali tif.

Priloge in slike morajo biti poimenovane z besedami, ki jih opredeljujejo, in arabskimi številkami (npr. Table 1:, Figure 1: itn.). Za dvopičjem sledi besedilo oziroma naslov. Vsi navedki (reference), citirani v besedilu, se morajo nanašati na seznam literature. V besedilu jih je treba oštevilčiti po vrstnem redu, po katerem se pojavljajo, z arabskimi številkami v oklepaju. Prvi navedek v besedilu opredeli številko oziroma vrstni red ustreznega vira v seznamu literature. Če se avtor v besedilu ponovno sklicuje na že uporabljeni vir, navede tisto številko, ki jo je vir dobil pri prvem navedku. Citirana so lahko le dela, ki so tiskana ali kako drugače razmnožena in dostopna javnosti. Neobjavljeni podatki, neobjavljena predavanja, osebna sporočila in podobno naj bodo omenjeni v navedkih ali opombah na koncu tiste strani, kjer so navedeni. V seznamu literature so viri urejeni po vrstnem redu. Če je citirani vir napisalo šest ali manj avtorjev, je treba navesti vse; pri sedmih ali več avtorjih se navedejo prvi trije in doda et al.

Da bi se morebitni popravki lahko objavili v naslednji številki, jih morajo avtorji pravočasno sporočiti glavnemu uredniku.

Načini citiranja

Knjiga: Hawkins JD. Gene structure and expression. Cambridge: University Press, 1991: 16.

Poglavje ali prispevek v knjigi: Baldessarini RJ. Dopamine receptors and clinical medicine. In: Neve KA, Neve RL, eds. The dopamine receptors. Totowa: Human Press, 1996: 475-98.

Članek iz revije ali časopisa: Fuji J, Otsu K, Zorzato F, et al. Identification of mutation in porcine ryanodine receptor asociated with malignant hyperthermia. Science 1991; 253: 448-51.

Članek iz zbornika referatov: Schnoebelen CS, Louveau I, Bonneau M. Developmental pattern of GH receptor in pig skeletal muscle. In: the 6th Zavrnik memorial meeting. Lipica: Veterinary Faculty 1995: 83-6.

Slov Vet Res 2010; 47 (4)

Original Scientific Articles

Proceedings of 1. Workshop Understanding Cell Communication using Modern Microelectronics