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$Ca_V1.3$ and BK Channels for Timing and Regulating Cell Firing

David Henry Vandael · Andrea Marcantoni · Satyajit Mahapatra · Anton Caro · Peter Ruth · Annalisa Zuccotti · Marlies Knipper · Emilio Carbone

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Abstract L-type Ca^{2+} channels (LTCCs, Ca_V1) open readily during membrane depolarization and allow Ca^{2+} to enter the cell. In this way, LTCCs regulate cell excitability and trigger a variety of Ca^{2+}-dependent physiological processes such as: excitation–contraction coupling in muscle cells, gene expression, synaptic plasticity, neuronal differentiation, hormone secretion, and pacemaker activity in heart, neurons, and endocrine cells. Among the two major isoforms of LTCCs expressed in excitable tissues ($Ca_V1.2$ and $Ca_V1.3$), $Ca_V1.3$ appears suitable for supporting a pacemaker current in spontaneously firing cells. It has steep voltage dependence and low threshold of activation and inactivates slowly. Using $Ca_V1.3^{-/-}$ KO mice and membrane current recording techniques such as the dynamic and the action potential clamp, it has been possible to resolve the time course of $Ca_V1.3$ pacemaker currents that regulate the spontaneous firing of dopaminergic neurons and adrenal chromaffin cells. In several cell types, $Ca_V1.3$ is selectively coupled to BK channels within membrane nanodomains and controls both the firing frequency and the action potential repolarization phase. Here we review the most critical aspects of $Ca_V1.3$ channel gating and its coupling to large conductance BK channels recently discovered in spontaneously firing neurons and neuroendocrine cells with the aim of furnishing a converging view of the role that these two channel types play in the regulation of cell excitability.

Keywords L-type calcium channels · Pacemaking currents · Action potential firing · Neurons · Chromaffin cells

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Introduction

Spontaneous electrical activity in excitable cells is fundamental to the control of basic physiological functions. Usually, spontaneous firings originate in narrow areas and spread to neighboring cells to drive rhythmic patterns of activity such as circadian rhythms in the central nervous system, activity-dependent hormone release in neuroendocrine cells, and heart beating [1, 2]. To undertake these duties, spontaneously firing cells are equipped with a set of ion channels that turn on at subthreshold membrane potentials and carry sufficient inward current to drive the action potential (AP) upstroke [3, 4]. In many cells, a spontaneous subthreshold depolarization is associated with Na^+ entry through either hyperpolarization-activated cation channels (HCN) or voltage-gated Na^+ channels [5]. There is, however, an increasing number of neurons and neuroendocrine cells in which subthreshold Ca^{2+} channels are shown to contribute to pacemaking. Besides the low voltage-activated T-types that are by definition sub-

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Nimodipine inhibits AP firing in cultured hippocampal neurons predominantly due to block of voltage-dependent potassium channels

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Abstract. L-type calcium channels (LTCC) are important functional elements of hippocampal neurons contributing to processes like memory formation and gene expression. Mice lacking the $Ca_V1.2$ channel in hippocampal pyramidal cells exhibited defects in spatial memory (Moosmang et al. 2005) and lowered frequency of repetitive action potential (AP) firing (Lacinova et al. 2008). We tested the contribution of LTCC to AP firing of cultured rat neonatal hippocampal neurons using the dihydropyridine channel blocker nimodipine. Ionic currents and APs were recorded in the whole cell patch clamp configuration. A prolonged depolarizing current pulse activated the firing of a series of APs. The presence of 10 μM nimodipine blocked all but the first AP in series. This concentration, which is potent enough to completely block LTCC, inhibited about 35–50% of the total calcium current. In addition, nimodipine blocked about 50% of both calcium-dependent and voltage-dependent potassium currents whereas the sodium current was not affected. We suggest that nimodipine suppressed the firing of APs in cultured neonatal rat hippocampal neurons due to inhibition of both calcium and potassium currents.

Key words: Hippocampal neurons — Nimodipine — Potassium current — Calcium current — Action potential

Abbreviations: AP, action potential; DHP, dihydropyridine; LTCC, L-type calcium channel.

Introduction

The hippocampus is a brain region participating in the formation of explicit memory, i.e. memory of facts and places (reviewed in Kandel 2009). The decisive element of memory forming is an activity-dependent strengthening of synaptic transmission, the so-called long term potentiation (LTP) (Bliss and Lomo 1973). Two phases of LTP can be distinguished: an early phase requiring covalent protein modification and a late protein synthesis-dependent phase (Frey et al. 1988). L-type calcium channels (LTCC) contribute to several memory-forming pathways, e.g., the cAMP response element-binding protein pathway (Deisseroth et al. 2003), Hebbian synaptic plasticity (Grover and Teyler 1990; Bauer et al. 2002; Moosmang et al. 2005) or transcription of brain-derived neurotrophic factor (Murphy et al. 1991; West et al. 2001).

The prevalent LTCC isoform expressed in hippocampus is the $Ca_V1.2$ isoform (Hell et al. 1993; Sinnegger-Brauns et al. 2004). Absence of the $Ca_V1.2$ channel in adult mouse hippocampus resulted in defects of late phase LTP and deficits of spatial memory (Moosmang et al. 2005). LTCC may support LTP and consequently memory forming through back-propagated somatic action potentials (APs) (Spruston et al. 1995; Hoffman et al. 1997; Kampa et al. 2006). Somatic APs alone are sufficient to activate mechanisms supporting late-phase LTP and to convert early phase LTP into stable late LTP (Dudek and Fields 2002). Therefore the contribution of LTCC to shaping AP trains is of major interest.

Reported effects of dihydropyridine (DHP) LTCC blockers on hippocampal excitability are controversial. In rabbit CA1 pyramidal hippocampal neurons, nimodipine increased excitability by increasing the number of APs and by decreasing accommodation frequency during a prolonged depolarizing cur-

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A novel bioelectronic glucose sensor to process distinct electrical activities of pancreatic beta-cells *

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Yannick Bornat, Sylvie Renaud, Member, IEEE, and Jochen Lang, Member, IEEE

Abstract — **Glucose sensors have improved and facilitated therapy for type 1 diabetes. However, they are still not capable to sense all physiological signals and to act in a closed-loop. Pancreatic β-cells have been shaped during evolution as biological sensors and offer the advantage to integrate all physiological signals in addition to glucose. Moreover, biosensors based on these cells may also serve for non-invasive and continuous long-term characterization of β-cells, drug research, tissue engineering and pre-transplantation quality control. β-cells alter their electrical activity upon exposure to glucose and physiological hormones and we have used these properties to design a biosensor. To this end signals were recorded extracellularly from islet cells kept on multi-electrode arrays. Slow and rapid oscillations were observed, both modulated by glucose. Especially slow oscillations are very robust and have an excellent signal/noise ratio. Signal processing functions were designed to separate the two activities to extract and analyze relevant parameters. These parameters correlate very well with either increasing or decreasing glucose concentrations. An electronic device is under construction, based on an embedded FPGA capable of processing multiple channels in parallel. In the future, such a device shall be used as a portable real-time biosensor regulating insulin delivery from a pump.**

I. Introduction

Type I diabetes is a chronic disease of increasing incidence [1] with loss of pancreatic β-cells, that normally produce and secrete insulin in response to nutrients such as glucose. Therapy implies determination of blood glucose levels and corresponding injections of the potent hormone insulin. More recently transplantation of islets has been established in the case of severe and difficult to control type I diabetes. Considerable hope is placed in differentiation of stem cells though successful protocols are still not available [2].

The introduction of electrochemical glucose sensors has presented a major breakthrough in diabetes therapy [3]. The continuous monitoring of glucose levels and injection of the corresponding amounts of insulin thus became feasible. However, this approach captures only part of the information the organism uses for physiological regulation of homeostasis. Within the body a group of specialized cells, the so-called α-, β- and δ-cells, are arranged in a micro-organ to sense the nutrient levels and demand in the hormone insulin. Biological sensors, such as pancreatic islets, have been shaped during half a billion years of evolution and in contrast to currently available medical devices, they rely on a number of different relevant signals to measure a variety of food-derived energy and to account also for the state of the entire organism and its requirements. Thus insulin need is signaled not only by glucose but also and by nutrients other than glucose, such as lipids and certain amino acids, and by several hormones, such as GLP-1 or GIP [4].

The integration of information about the presence of these molecules by pancreatic β-cells subsequently provides a precisely tuned signal to regulate cellular actions, such as the release of the potent hormone insulin into the bloodstream. Islet cells, including β-cells, are excitable cells similar to nerve cells. They relate signals by changing their electrical

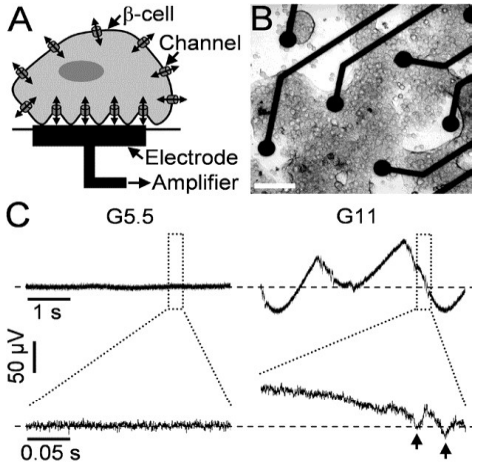

Fig. 1: Extracellular recording of electrical signals from mouse islet cells cultured on multi-electrode arrays (MEA). A, Scheme of electrical signals generated by the activity of ion channels in a β-cell cultured on a microelectrode connected to an amplifier. B, 2-days-old culture of mouse islet cells growing on the MEA titanium nitride electrodes (planar, 30 µm diameter; scale bar: 100 µm). C, representative electrophysiological recordings obtained from one electrode of the MEA in B. The null-voltage is indicated by horizontal broken lines. At low glucose concentration (5.5 mM, G5.5) electrical oscillations are absent (left), whereas a concentration mimicking hyperglycaemia (11 mM, G11) induced low frequency oscillations (right, upper trace) on which rapid signals (action potentials) are grafted as can be seen at higher temporal resolution (see arrows at right, lower trace).

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Vápnikové prúdy v diferencovaných a nediferencovaných PC12 bunkách

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Úvod a formulácia cieľa

Bunková línia PC12 predstavuje nesmrteľnú líniu z rakovinového nádora phaeochromocytómu, izolovaného z adrenálnej meduly potkanov [1]. PC12 bunky prvýkrat izolovali Greene a Tischler v New England Hospital Deaconess v roku 1976 z transplantovateľného feochromocytomového nádora [1].

Pri kultivácii za normálnych podmienok sú PC12 bunky morfologicky, fyziologicky a biochemicky podobné chromafínovým bunkám (polygonálne alebo guľaté bez alebo s veľmi krátkymi výbežkami), avšak po pridaní NGF (neuronal grown factor, neuronálny rastový faktor) sa začínajú morfologicky a funkčne diferencovať na neuróny a vysielaním neuritových výbežkov nadobúdajú sympathetický neuronálny fenotyp [2], začínajú prisadať na dno a predlžovaním sa zmenia na trojhranné alebo oválne tvarované kužely [3].

Neuronálny rastový faktor - NGF predstavuje potrebný aktivátor Ras/MAP kinázovej dráhy [4]. Diferenciácia a následné fyziologické a biochemické zmeny aktivované NGF sú najvyužívanejšie pri použití tejto bunkovej línie na štúdium mechanizmu neurálnej diferenciácie [1]. PC12 tiež predstavujú model na štúdium zmien, ktoré sú ťažko pozorovateľné v bunkách, ktoré sú už funkčne diferencované na neuróny [5].

Bunková línia PC12 predstavuje tiež vhodný modelový objekt na štúdium vápnikových kanálov. Vápnikové kanály umožňujú transport Ca^{2+} iónov z jednej strany membrány na druhú a plnia funkciu prenášačov elektrického signálu potrebného pre mnohé vnútrobunkové procesy [6].

Napäťovo závislé vápnikové kanály (VGCC) predstavujú veľkú rodinu integrálnych membránových proteínov. Kontrolujú selektívny vtok Ca^{2+} iónov po zmene membránového potenciálu [7]. Molekulárnym klonovaním cDNA sa podarilo identifikovať 10 génov pre α_1 podjednotku, ktoré sú rozdelené do troch skupín: L, N a T [8].

Vápnikový prúd tečúci cez vápnikový kanál L-typu je hlavným iniciátorom neurosekrécie a podieľa sa na nervovej excitabilite [5], spúšťa signalizačné procesy vedúce ku kontrakcii buniek, v neurónoch sa podieľa na regulácii génovej expresie [9], kontroluje procesy

А.Г8.4
Calcium Currents in Differentiated and Undifferentiated PC12 Cells

Summary

The PC12 cell line, derived from rat adrenal pheochromocytoma, serves as a valuable model for research on neuronal differentiation and calcium channel function. Under normal conditions, these cells resemble chromaffin cells but differentiate into neuron-like cells with neurites when treated with nerve growth factor (NGF), activating pathways like Ras/MAP kinase.

This study compared calcium currents in untreated and NGF-treated PC12 cells using patch-clamp techniques. In undifferentiated cells, only 32% showed measurable calcium currents, whereas NGF treatment raised this to 70%. NGF also caused cells to stop dividing, grow larger, and express more voltage-gated Ca^{2+} channels, though current density remained stable due to increased cell size. These findings highlight PC12 cells as an effective model for studying neural differentiation and ion channel regulation.

Elektrofyziologické zmeny $Ca_v1.2$ v PC12 bunkách pri časovom ovplyvňovaní neuronálnym rastovým faktorom NGF

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Abstrakt

PC12 bunky predstavujú excelentný model na štúdium neuronálnej diferenciácie aj na štúdium vápnikových kanálov. Pri ovplyvnení PC12 buniek neuronálnym rastovým faktorom dochádza k ich funkčnej diferenciácii na neurónom podobné bunky. V našej práci sme zisťovali ako sa morfologická zmena PC12 buniek prejaví na fyziologickej úrovni. Sledovali sme ako sa zmení veľkosť prúdu, ktorý preteká cez $Ca_v1.2$ vápnikový kanál. Prechod iónov cez kanály sme merali metódou patch clamp.

Kľúčové slová: *PC12*; *napäťovo-závislé vápnikové kanály*; *$Ca_v1.2$*; *NGF*; *patch clamp*

Úvod a formulácia cieľa

Na štúdium napäťovo závislých vápnikových kanálov je dlhé roky používaná línia PC12 buniek. Imortalizovaná línia bola prvýkrát izolovaná Greenom a Tischlerom z rakovinového nádora feochromocytómu z nadobličky potkanov [1]. Okrem štúdia iónových kanálov je používaná pri štúdiu chemických procesov spojených so syntézou, akumuláciou a uvolňovaním neurotransmiterov [2], na neurochemické štúdie [3], a na výskum selektívnej expresie génov pre iónové kanály [4]. PC12 bunky sú vhodné na aj skúmanie neuronálnej diferenciácie a bunkovej proliferácie [5]. Diferenciácia PC12 buniek je navodená neuronálnym rastovým faktorom, ktorý aktivuje Ras/MAP kinázovú dráhu [6]. Pridanie neuronálneho rastového faktora podnecuje PC12 bunky k funkčnej a morfologickej diferenciácii na neurónom podobné bunky [7]. V cytoplazmatickej membráne natívnych PC12 buniek sa vyskytuje viac typov ligandovo- a napäťovo-závislých iónových kanálov, ako acetylcholínom aktivované kanály alebo napäťovo závislé kanály (sodíkové, vápnikové a draslíkové) [4]. V našej práci sme sa zamerali na napäťovo závislé vápnikové kanály.

Napäťovo závislé vápnikové kanály prvýkrát popísali Paul Fatt a Bernard Katz [8]. Tento typ kanálov zabezpečuje prenos Ca^{2+} iónov z extracelulárnej strany membrány do vnútra bunky a zároveň prenáša elektrický signál nevyhnutný pre mnohé vnútrobunkové procesy. Prúd tečúci cez $Ca_v1.2$ sa podieľa na excitácii a kontrakcii svalov a sekrécii inzulínu, nachádza sa v svaloch, hladkých, priečne pruhovaných aj v srdcovej svalovine [9]. $Ca_v1.2$

А.Г8.5

Electrophysiological Changes of Cav1.2 in PC12 Cells During Temporal Modulation by Neuronal Growth Factor NGF

Summary

PC12 cells represent an excellent model for studying neuronal differentiation as well as for investigating calcium channels. When PC12 cells are influenced by neuronal growth factor, they undergo functional differentiation into neuron-like cells. In our work, we investigated how the morphological changes of PC12 cells manifest at the physiological level. We observed how the current passing through the Cav1.2 calcium channel changes. The ion flow through the channels was measured using the patch clamp technique.

Заключение. Проведеното изследване на основа посещаемостта на спортните площадки на ЮЗУ "Неофит Рилски", разкрива позитивните тенденции в мотивацията на студентите да ползват спортните площадки, които вероятно са свързани с ролята на спортните педагози в Университета, както и осигурените възможности за безплатно ползване на игрищата на университетския спортен комплекс.

Ключови думи: спортни площадки, студенти, посещаемост, спортни интереси.
Key words: sports grounds, students, attendance, sports interests.

Литература

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СОМАТОТИПИРАНЕ: КЛАСИФИКАЦИИ И МЕТОДОЛОГИЯ

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Въведение. От момента на възникване на цивилизацията до ден днешен, човекът търси взаимовръзка между формата или телосложението и съдържанието, което може да обхваща различни страни от физическите, психическите и когнитивните способности на индивида и начинът по който си взаимодейства със социума, което формира неговия темперамент. Още от древността тази връзка е забелязана от различни учени, които разработват класификации и терминология, описващи и свързващи нашите психични качества с телосложението и конституцията ни. Повечето класификации днес имат историческо значение, но продължават да са широко използвани в различни сфери на познанието, изкуствата и обществения живот. От тях, съвременно приложение при научни изследвания намира предимно класификацията на Heath и Carter (1967). Тя описва телосложението на човека комплексно като комбинация от три основни соматотипни компонента.

Цел. Настоящата теоретична разработка цели да представя хронологично еволюцията на соматотипирането от Хипократ до наши дни, както и значението и спецификата на съвременната методология за определяне на основните соматотипове.

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ПОДОБРЯВАНЕ НА ЗАПЛОДЯЕМОСТТА НА КРАВИ И БИВОЛИЦИ, ТРЕТИРАНИ СЛЕД ОСЕМЕНЯВАНЕ С DIFUROL-1 ИЛИ DIFUROL-2

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Резюме

Целта на проведените изследвания е да се установи сравнително терапевтичният ефект от извършеното протектирано осеменяване чрез Difurol-1 и Difurol-2 при крави и биволици.

За прецизиране подбора на опитните животни използвахме предложената от Флегматова биологична проба, с която диагностицирахме скритият еднометрит. На 24-тия h след заплождането (осеменяването) извършихме интраутеринно впръскване на Difurol-1 и Difurol-2, а в контролната група използвахме изотоничен разтвор (0,9% NaCl).

Препаратите Difurol-1 и Difurol-2, приложени еднократно интраутеринно на 24-тия h след осеменяването при крави, повишават заплодяемостта на 66,6% до 90%, а при контролите — 33,3% до 40%.

Препаратите Difurol-1 и Difurol-2, приложени еднократно интраутеринно на 24-тия час след осеменяването при биволици, повишават заплодяемостта до 83,3%, при контролите — 50%, което е показател за еквивалентна клинична терапевтична ефективност на двата препарата.

FERTILITY IMPROVEMENT IN COWS AND BUFFALO COWS TREATED AFTER INSEMINATION WITH DIFUROL-1 OR DIFUROL-2

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Summary

This study was conducted to investigate the influence of the intrauterine antibiotic treatment on the conception rate of cows and buffaloes inseminated artificially.

The study population comprised 44 Holstein cows and 18 buffaloes (Murrha x Bulgarian Buffalo). The cows were separated into two groups: industrial farm cows and single- family farm cows. Each group was divided into three subgroups: the first one was treated intrauterine with Difurol-1; the second group was treated intrauterine with Difurol-2; the third one was a control group, treated intrauterine with 20 ml isotonic NaCl.

The buffaloes were separated into three subgroups. Those in group 1 were treated intrauterine with Difurol-1,

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СРАВНИТЕЛНО ПРОУЧВАНЕ НА ТЕРАПЕВТИЧНИЯ ЕФЕКТ НА DIFUROL–1 И DIFUROL–2, ПРИЛОЖЕНИ ЗА ЛЕКУВАНЕ НА ХРОНИЧНИ ЕНДОМЕТРИТИ ПРИ КРАВИ

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Резюме

С изследванията се целеше да се установи терапевтичната ефективност на разработените във фирма „Балканфарма“ АД , Разград, интраутеринни суспензионни лекарствени форми Difurol–1 (Tetracycline hydrochloride, екв. на 500 mg, Clioquinol, екв. на 500 mg и помощни вещества до 20,0 g) и Difurol–2 (Tetracycline hydrochloride, екв. на 500 mg, Clioquinol, екв. на 500 mg, Tylosin base екв. на 500 mg и помощни вещества до 20,0 g), предназначени за лекуване на различни клинични форми на хронични ендометрити при крави.

При опитните крави, болни от *endometritis catarrhalis chronica*, след третирането им с Difurol–1 и Difurol–2, клинично бяха излекувани по 83,3 %, докато в контролната група, третирани с луголов разтвор, те са 50,0 %. От кравите с *endometritis catarrhalis purulenta chronica* процентът на клинично излекуваните след интраутеринното впръскване на Difurol–1 бе 66,7 % , а с Difurol–2 – 83,3 % , а от третираните с луголов разтвор положителен клиничен ефект беше получен само при 50,0 % от животните. От болните с *endometritis purulenta chronica* крави, след лекуване с Difurol–1 бяха оздравели 50,0 %, докато при тези, третирани с Difurol–2, процентът на излекуваните бе 66,7 %. В контролната група клинично оздравяване бе регистрирано при 33,4 % от животните.

Ключови думи: Difurol–1, Difurol–2, крави, интраутеринно приложение, хронични ендометрити.

COMPARATIVE STUDY OF THE THERAPEUTIC EFFECT OF DIFUROL–1 AND DIFUROL–2 ON CHRONIC ENDOMETRITIS IN COWS

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Summary

The aim of the study was to examine the therapeutic effect of suspensions for intrauterine application manufactured by Balkanfarma – Razgrad – Difurol–1 (Tetracycline hydrochloride – 500 mg, Clioquinol – 500 mg and excipiente ad 20,0 g) and Difurol–2 (Tetracycline hydrochloride – 500 mg, Clioquinol – 500 mg, Tylosin base – 500 mg and excipiente ad 20,0 g), developed for treatment of different clinical forms of chronic endometritis in cows.

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ПРОУЧВАНИЯ ВЪРХУ ЛОКАЛНАТА И ОБЩАТА ПОНОСИМОСТ НА ДВА ЛЕКАРСТВЕНИ ПРОДУКТА - DIFUROL-1 И DIFUROL-2, ПРЕДНАЗНАЧЕНИ ЗА ИНТРАУТЕРИННА ТЕРАПИЯ НА ХРОНИЧНИ ЕНДОМЕТРИТИ ПРИ КРАВИ И БИВОЛИЦИ

ЖЕКО БАЙЧЕВ[1], ПЪРВАН ПЪРВАНОВ[1], СЛАВОМИР СИМЕОНОВ[1], ТАНДЖУ МЕХМЕДОВ[1], АНТОН КАРО[1], КАЛОЯН ТРАЙКОВ[1], ГЕОРГИ ЛЕОНОВ[1], ДРАГОМИР ИВАНОВ[1], ПАСКАЛ ЖЕЛЯЗКОВ[2], БОРИС ДОНЕВ[2], ВЛАДИ КИРИЛОВ[2]
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Резюме

Проучена е локалната и общата поносимост на препаратите Difurol–1 и Difurol–2, произведени в Балканфарма – Разград.

Получените резултати след интраутеринното впръскване на препаратите в двойна доза (2 спринцовки) на крави, болни от хроничен катарално-гноен ендометрит показват, че е налице локална и обща поносимост. Третираните крави не са показали признаци на възпаление, странични реакции, като депресия, болка, контракции и евакуация на лекарствените вещества. Не са настъпили промени в ректалната температура, пулсовата и дихателна честота, руменовите движения, общия протеин, уреята и др. биохимични показатели. При третираните здрави крави и биволици с единични дози от посочените препарати не се наблюдават промени в изследваните показатели.

Интраутеринното впръскване на Difurol–1 и Difurol–2 при крави и биволици не предизвиква признаци на локална и обща непоносимост.

STUDIES ON THE LOCAL AND TOTAL TOLERANCE OF DIFUROL-1 AND DIFUROL-2 USED FOR MEDICATION OF CHRONIC ENDOMETRITIS IN COWS AND BUFFALO COWS

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Summary

The present studies examine the local and total tolerance of Difurol–1 and Difurol–2, manufactured by Balkanfarma-Razgrad.

The results, compared after intrauterine application of Difurol–1 and Difurol–2 in double doses (2 syringes) in cows with endometritis catarrhalis purulenta chronica, show that they are locally and totally well tolerated by the cows. The treated cows showed no signs of inflammation or side effects such as depression, pain, contraction, evacuation of the medicine. There was no change in the rectal temperature, pulse, respiration, rumenal movements, total protein, urea or other biochemical parameters. The same results were obtained when treating healthy cows and buffalo cows with single doses of Difurol–1 and Difurol–2.

The intrauterine treatment of cows and buffalo cows with Difurol–1 and Difurol–2 does not bring about local or total intolerance.

ВЪЗРАСТОВИ ПРОМЕНИ В СОМАТОТИПНИТЕ КОМПОНЕНТИ ПРИ МЛАДИ ЖЕНИ

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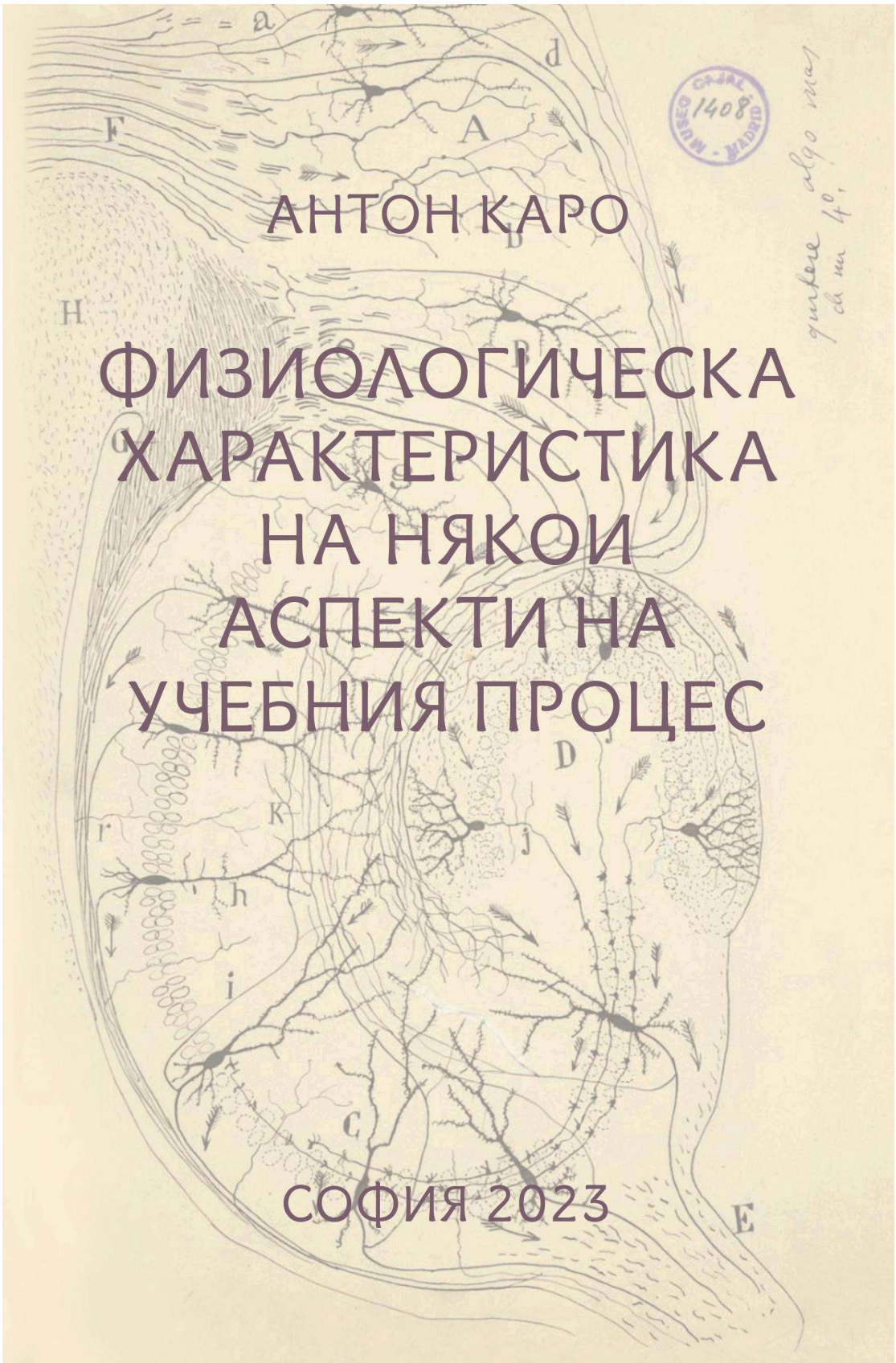
Въведение. Соматотипирането е съвременен подход за комплексна количествена оценка на телосложението и състава на телесната маса, разработен от Heath & Carter (1967), чрез измерване на антропометични показатели, кожни гънки и изчисляване на три соматотипни компоненти (СК): - ендоморфен компонент (ЕНК), отразяващ участието на мастната тъкан; - мезоморфен компонент (МК), представящ скелетната мускулатура; и – ектоморфен компонент (ЕКК), свързан с т.нар. линеарност на тялото. Соматотипирането се прилага при оценяване на промените в СК в хода на развитието, израстването, възрастови изменения, ролята на генетични фактори (Peeters et al., 2007) и начина на живот, фактори на околната среда, влияние на хронични заболявания и пр. В литературата са представени доказателства за динамиката в СК при различни спортни дисциплини, като белег за адаптационни изменения и ниво на тренираност (Stewart et al., 2003; Leake & Carter, 2007; Malousaris et al., 2008; Mielgo-Ayuso et al., 2017), но такива изследвания върху нетренирани жени са бегло представени. Спецификата в конфигурацията на женското тяло, като конус с основата надолу, е част от интереса към подобни проучвания. Базирайки се на изложеното, обект на настоящото проучване е динамиката в СК при млади жени.

Цел. Да се определят СК при млади нетренирани жени между 18 до 29 години, да се потърси динамиката в СК, като се обособят две възрастови подгрупи и да се изчислят корелационни зависимости между СК и някои основни антропометрични показатели.

Методи. Експериментите бяха одобрени от Университетската комисия по етика на научните изследвания. В изследването участваха жени, които не се занимават системно с физическо натоварване, на възраст от 18 до 29 години. След запознаване с дизайна и методологията на измерванията, лицата подписаха декларации за информирано съгласие и бяха обособени в две възрастови подгрупи: от 18 до 21 години (n=28); и от 22 до 29 години (n=21). За определянето на соматотипа бе приложен метода на Heath & Carter, описан от Duquet & Carter (2009). Измерванията обхващаха следните параметри: телесна маса (kg), ръст (cm), две обиколки в cm (на мишница и прасец), две широчини в cm (на фемур и хумерус) и три кожни гънки в mm (на трицепс, субскапуларна и супраспинална). За изчисляване на СК са приложени съответни регресионни уравнения. Стойностите на координатите на точките, представящи графично соматотипа в т.нар. соматограма, се изчисляваха както следва: - за оста *Ox* като разлика между ЕКК и ЕНК; - за оста *Oy* като разлика между удвоеното произведение на МК и сумата между ЕНК и ЕКК. За всяко лице бяха изчислени СК и координатите. Осреднените стойности на СК и на посочените координати, както и на всички останали параметри за двете възрастови групи, бяха изчислени с дискриптивна статистика и представени като $\overline{X} \pm SD$. Наличието на статистически значими различия между средните стойности на измерените параметри на двете възрастови подгрупи, беше тествано с непараметричен тест на Mann – Whitney ($p<0.05$). Коефициентът на Pearson ($p<0.05$) беше изчислен с



АНТОН КАРО

ФИЗИОЛОГИЧЕСКА ХАРАКТЕРИСТИКА НА НЯКОИ АСПЕКТИ НА УЧЕБНИЯ ПРОЦЕС

СОФИЯ 2023

Антон Каро

Физиологическа характеристика на някои аспекти на учебния процес

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СЪДЪРЖАНИЕ

„Главното изискване в учебния процес е разбирането и творческото решаване на нови задачи. Но главната спънка в това отношение е запаметяването и автоматизацията на материала, за да може след това на тази база да се подава нова информация и да се развиват нови умения. Не запаметяването е основното в обучителния процес, а създаването на мислене, съответстващо на придобитата квалификация, но поради това, че то се явява ограничаващ фактор за създаването и разгръщането на това мислене, ролята му се преекспонира.“ – Г. Лозанов (1975).

„Любящите родители, добрите учители, призвани да предадат на следващите поколения всичко полезно, което са успели да възприемат от предишните поколения, както и натрупания житейски опит, съзнавайки какъв огромен обем информация трябва да усвоят техните възпитаници, допринасят за стесняване на каналите за изучаването на света. Те несъзнателно внушават на децата собствения си страх от ограничените човешки възможности, от осъзнаването и възприемането на реалността, от трудността да се справят с препятствията.“ – Р. Начева, Л. Гановски, З. Думева (2012).

ВЪВЕДЕНИЕ

От момента на възникване на човешката цивилизация до наши дни предаването на знания и умения от едно поколение на друго се явява основен компонент на учебния процес. Днес, в епохата на информационната революция, обемът на знанията и обхватът на уменията, необходими за успешно участие в обществото, са на безпрецедентно високо ниво. Повишените изисквания се явяват индикатор за необходимост от поява на нови педагогически подходи.

Тази монография се стреми да проучи някои физиологически аспекти на учебния процес и тяхната интеграция в съвременните методи на обучение. Представените в нея анализи и илюстративни разработки са насочени към оптимизиране на учебния процес и подпомагане на преподавателите, но също така и на обучаемите, независимо дали ученици или студенти, в успешното им справяне с нарастващите предизвикателства, които стоят пред учебния процес.

Общоприето е да се смята, че учебният процес представлява систематична и организирана дейност, насочена към предаване, придобиване и усвояване на знания, умения и компетентности, като стимулира критичното мислене, развитието на творческите способности, формирането на социални умения и подготвя индивидите за активно участие в обществото.

В днешния образователен контекст, обаче, акцентът често пада върху когнитивните и информационни аспекти на учебния процес, често се пропускат физиологичните процеси, стоящи в основата на емоциите, мотивацията, поведението, обучението, паметта, които имат също толкова важно влияние върху учебния успех.

Тази монография систематизира физиологичните аспекти, като разглежда учебния процес чрез призмата на ученията на Павлов за условния рефлекс, на тезата за емоцията на очакването на Бехтерев и на принципи от интегралната психотерапия с приложение в педагогиката, формулирани от Лозанов. Обръща се специално внимание на важността на взаимодействието между психическите и физиологичните процеси в учебния процес като неотделима част от създаването на хармонична и балансирана образователна среда.

Монографията има за цел да обогати съществуващите представи за учебния процес и да допринесе за по-ефективно усвояване на знания и умения. Чрез тази монография се предлагат решения на някои фундаментални препятствия, свързани с липсата на разбиране за стойността на изучавания материал и неговото непосредствено практическо приложение в живота, които често съпътстват учебния процес и водят до ниски образователни резултати. Анализирането на въздействието на физиологичните фактори върху учебния успех може да доведе до формиране на нов тип отношение към учебния процес от страна на обучаемите, до по-добро разбиране на индивидуалните им потребности и структуриране на образователните методи.

Подобаващото включване на физиологическите аспекти в учебния процес може да допринесе за създаването на нова образователна парадигма. Тя може да промени начина, по който възприемаме, интерпретираме, разбираме и практикуваме образованието, като подчертава интегративния характер на човешката психофизиология и влиянието ѝ върху постигането на желани образователни резултати.

Монографията е насочена към колегите физиолози, към педагозите, учителите, университетските преподаватели, но също така и към родителите, които търсят нов подход към своите деца в информационната ера.

Монографията има за цел да обогати съществуващите представи за учебния процес и да допринесе за по-ефективно усвояване на знания и умения. Чрез тази монография се предлагат решения на някои фундаментални препятствия, свързани с липсата на разбиране за стойността на изучавания материал и неговото непосредствено практическо приложение в живота, които често съпътстват учебния процес и водят до ниски образователни резултати. Анализирането на въздействието на физиологичните фактори върху учебния успех може да доведе до формиране на нов тип отношение към учебния процес от страна на обучаемите, до по-добро разбиране на индивидуалните им потребности и структуриране на образователните методи.

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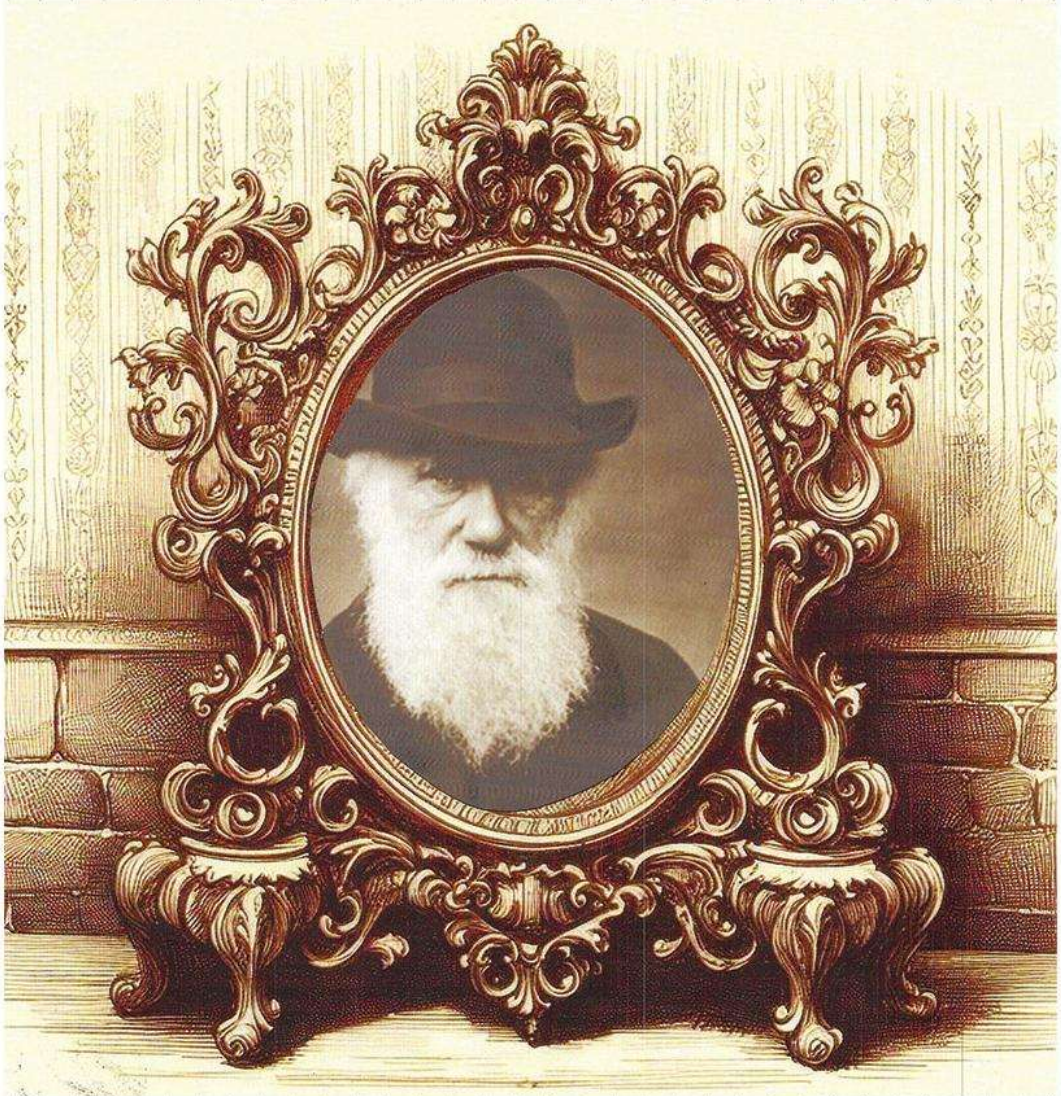

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CONTENT

FOREWORD

This monograph connects the evolution of the brain in mammals, the development of intelligence in animals and humans, and the role of emotions in both animal and human life. The author's contributions to the field are both original and practical, providing readers with not only knowledge but also tools for personal growth and emotional well-being. The readers may acquire a new perspective to a well-known theme and obtain a deeper understanding of what it is to be a human. Readers may also experience the interconnection of emotions, secretion of biologically active substances to which people may become addicted and then to transpose to patterns of acquired reflexes (learned behaviors) and how our emotions, shaped by the evolution of our brain and intelligence, influence our lives in ways we are only beginning to fully appreciate.

From the moment life emerged in the oceans, few evolutionary steps have been so transformative as the evolution of the brain. The development from simple neural tube to the complex organ that houses thoughts, emotions, and underlie certain patterns of behavior is significant. The presented monograph examines this evolutionary pathway, emphasizing the interconnection between brain development and emotions that are often described in literature as powerful and overwhelming forces that significantly shape our existence.

The author describes brain evolution in mammals with an emphasis on the limbic system, the part of the brain responsible for processing of emotions, which evolved as critical survival mechanisms. As the brain evolved, its capacity to process emotions increased. At the time emotions served as an early detection system (indicators) for potential threats and opportunities. The evolution of brain would also mean that emotions followed the steps to increase their number, as well as their complexity, for better adaptation to the ever changing environment. Some emotions would increase the chances for survival, while others would promote social interaction and increase the quality of life within the population. According to the author without the evolution of the brain, not only our cognition, but also our capacity for emotions would not exist the way we know it today. A high probability exists that with lower capacity for emotions our civilization would not be the same.

Brain evolution is linked to intelligence. The author guides us through the evolution of intelligence as an essential component of social interactions in non-human and human animals. Intelligence is defined not merely as a tool for problem-solving or abstract thinking, but as a fundamental skill for social interaction within the population. The ability to understand, predict, and influence the behavior of others has always been crucial for survival in social species, and humans are no exception. Of course, not every situation can be hold under control and the knowledge on emotions encompasses that notion.

This monograph pays special attention to the deep interconnection between intelligence and social emotions. The survival of our ancestors depended not only on their physical abilities but also on their capacity to adapt to life: to cooperate, to compete, and to form alliances. Social emotions such as empathy, pride, and guilt form social intelligence, which regulates interpersonal relationships and maintains social order. The author's exploration of this topic provides original and accessible interpretation to the readers by describing how emotions are connected to our evolutionary past.

Central to this observation is the author's original framework for understanding and managing emotions. There are 8 emotions that can be identified as basic, and every basic emotion may serve as a source for new emotions to emerge. Choosing one emotion for each basic emotion, the author created a set of 16 emotions altogether that are described not only as psychological states but also as indicators that can guide human behavior in specific contexts. The author reports that emotions act as internal compasses, pushing us towards actions that fulfill our needs and help us study, understand and manage the complexities of life. A possible example is fear that serves as alert to danger, prompting a fight-or-flight response, while joy signifies the completion of a specific task or more abstract needs that lead to wholeness.

The author continues further by identifying the fundamental needs that underlie the described emotions. By acknowledging, recognizing and understanding these needs, an individual can better understand why certain emotions arise and how they

can be effectively regulated. This framework offers valuable insights for both personal development and therapeutic practices, enabling individuals to identify and address the root causes of their emotional responses.

In addition to the work on emotions, the author also provides practical strategies for managing them, based on Tobin's theory. The coping strategies outlined in this monograph are presented through the author's distinctive perspective, offering fresh insights and clear definitions. Whether addressing problem-solving, seeking social support, or employing cognitive restructuring, the author presents these strategies with precision, using clear examples that make them accessible to a wide audience.

Another original contribution in this monograph is the tool designed for practicing emotional intelligence, organized as a set of cards for a game. This practical method not only makes the complex concepts of emotional intelligence more approachable but also offers an engaging and interactive way to apply these concepts in everyday life. The tool encourages participants to connect with their own emotions and those of others in a constructive manner, promoting a deeper understanding and mastery of emotional intelligence for a more examined life.

From an evolutionary standpoint, emotions, from the very beginning of mammal appearance on the map, have served as adaptive mechanisms, enhancing our ability to respond to environmental challenges and fostering social bonds.

These evolutionary advancements have not only aided in individual survival but also in the formation of complex social hierarchies in animals and humans.

Furthermore, emotional intelligence, encompassing the perception, understanding, and management of emotions, emerges as a crucial skill for life. The insights provided in this monograph emphasize that acknowledging the full spectrum of our emotions, both positive and negative, can lead to a more balanced life by understanding the physiological and psychological aspects of emotions.

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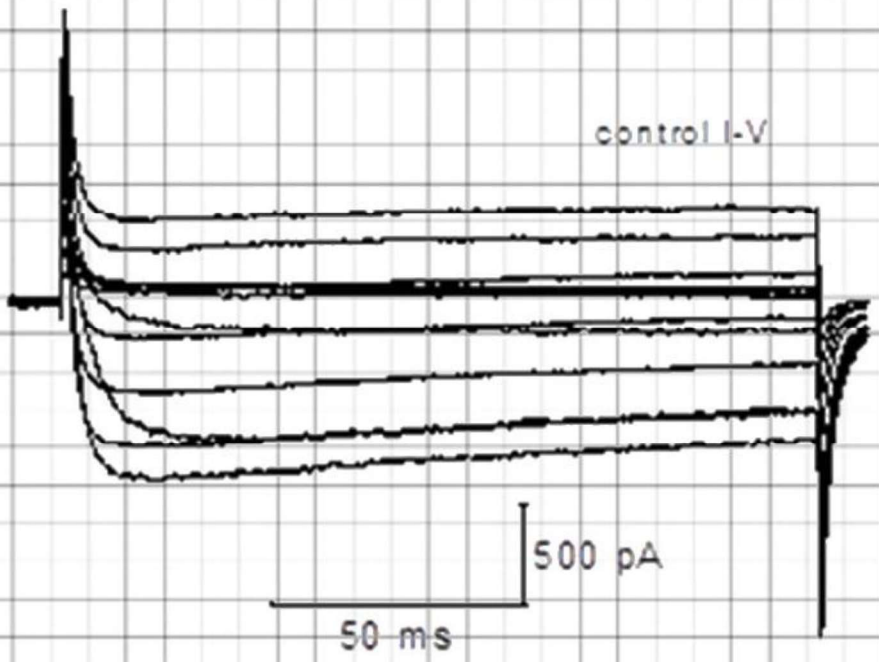
Anton Caro

Voltage-dependent calcium channels in rodent hippocampus



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FOREWORD

This book is based on a dissertation funded under Human resources and Mobility in the specific programme for research, technological development, and demonstration “Structuring the European Research Area” under the Sixth Framework Programme 2002-2006, part of the Marie Curie training network CAVNET.

The dissertation has three reviewers:

1. Prof. Alexandra Koschak from the Medical University in Vienna, Austria.
2. Prof. Jutta Engel from the University of Saarland in Homburg, Germany.
3. Prof. Jan Galik from the University in Kosice, Slovakia.

The dissertation was successfully defended on 15 March 2012 at the Institute of Molecular Physiology and Genetics in Bratislava, Slovakia.

CONTENT

ABSTRACT

L-type voltage-dependent calcium channels (LTCC) play an important role in memory formation and gene expression in the hippocampus. Our goal was to investigate the contribution of L-type calcium channels and more specifically of $Ca_V1.2$ channel to neuronal excitability. We evaluated and compared inward calcium currents, single action potentials and action potential series in presence and absence of LTCC. As experimental model we used primary culture of neonatal rat hippocampal cells. Channel activity was regulated by use of dihydropyridine L-type calcium channel blocker nimodipine, and by gene silencing of the gene for $Ca_V1.2$ channel by siRNA. Currents and action potentials were recorded in whole-cell patch clamp configuration. The presence of 10 μM nimodipine inhibited about 35 – 50 % of the total calcium current and blocked the generation of AP in series, induced by a prolonged depolarizing current pulse. It was unlikely that the latter effect could be attributed to block of LTCC only, therefore we examined possible effect of nimodipine on potassium and sodium conductances. Sodium current was not affected. Nimodipine blocked both calcium-activated and voltage-dependent potassium channels. Calcium-activated potassium channels

are coupled to voltage-dependent calcium channels. Therefore, observed inhibition could be indirect due to inhibition of calcium entry, or due to direct interaction of nimodipine with the channel. Voltage-dependent potassium channels were inhibited directly perhaps by an open channel block. Further, we studied the individual contribution of $Ca_V1.2$ channel by silencing corresponding gene by siRNA. We verified decreased level of mRNA for the $Ca_V1.2$ channel by RT-PCR and by functional test. $Ca_V1.2$ gene silencing significantly decreased the proportion of nimodipine-sensitive calcium current and the concentration of mRNA for the $Ca_V1.2$ channel. Pattern of firing series of action potentials and the basic electrophysiological parameters of neurons, like cell capacity and input resistance did not change.

L-type voltage-dependent calcium channels play an important role in memory formation and gene expression in the hippocampus. That is why we investigated their contribution and more specifically of $CaV_{1.2}$ channel to neuronal excitability.

We evaluated and compared inward calcium currents, single action potentials and action potential series in presence and absence of L-type voltage-dependent calcium channels using primary culture of neonatal rat hippocampal cells.

Channel activity was regulated by use of dihydropyridine L-type calcium channel blocker nimodipine, and by gene silencing of the gene for $CaV_{1.2}$ channel by siRNA. Currents and action potentials were recorded in whole-cell patch clamp configuration.

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THE PATCH CLAMP METHOD CONFIGURATIONS AND PROTOCOLS FOR ASSESSING NEURONAL EXCITABILITY

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ABSTRACT

Electrophysiology serves as an indispensable instrument in the investigation of the physiological and pathological characteristics of electrically active cells and their interconnected networks. The patch clamp method exhibits remarkable flexibility and can be employed in numerous configurations to examine a spectrum of properties.

However, adequate protocols are essential for precise measurements, ensuring the accuracy and reproducibility of experiments. The diversity of cell types requires tailored approaches, adding complexity and highlighting the need for robust protocols to enrich our understanding of neural functions.

The protocols proposed by the author represent a thoughtful contribution to the study of CA1 hippocampal neurons. They are designed to align with the physiological characteristics of these neurons, allowing sufficient time for cellular recovery following each electrical stimulus, avoiding artifacts caused by cellular fatigue. The protocols take into consideration the properties of the voltage-gated channels expressed, proving to be suitable for the study of neuronal excitability.

Key words: electrophysiology, excitability, patch clamp, input resistance, action potential.

Introduction

Nearly four decades ago, the advent of the patch clamp technique marked a significant breakthrough in Cellular Physiology and Biophysics by adding experimental value to the Hodgkin–Huxley model of the squid giant axon (Hodgkin et Huxley 1952) that earned its creators the Nobel prize in physiology in 1963, serving this way as a refinement of the voltage clamp (Neher et Sakmann 1976). This novel electrophysiological approach enabled scientists to observe the behavior of individual ion channels within the cell plasma membrane in their natural environment. Initially introduced for its ability to investigate single ion channels (Neher *et al.* 1978), the patch clamp method proved to be more versatile and powerful than anticipated, leading to the development of multiple configurations (Hamill *et al.* 1981). These configurations have profoundly enhanced our understanding of cellular electrical signaling, fundamentally transforming our knowledge of both traditionally excitable cells, such as neurons (Bean 2007; Santillo 2024) and muscle cells (Kornreich 2007), as well as other cell types such as chromaffin cells (Carbone *et al.* 2019), β-cells (Fridlyand *et al.* 2009) and even going beyond to in vivo recordings (Wang *et al*. 2016).

The patch clamp is an electrophysiological technique used to study the electrical properties of cells, cell membranes and occasionally isolated organelles. All patch-clamp configurations rely on a very high-resistance seal between a micropipette and a membrane. The seal is usually obtained by gentle suction. Erwin Neher, Bert Sakmann and Dieter Lux developed the patch clamp in the late 1970s and early 1980s (Hamill *et al.* 1981). Neher and Sakmann received the Nobel prize in physiology in 1991 for this work (Neher 1992).

To conduct the desired studies, adequate protocols are needed. These protocols ensure precision in measurements, which is critical for achieving accuracy and reproducibility in experiments.

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF DIFFERENTIATING NEONATAL RAT HIPPOCAMPAL NEURONS

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ABSTRACT

This study investigates the differentiation and electrophysiological properties of neonatal rat hippocampal CA1 neurons cultured in vitro. Primary hippocampal neurons were extracted from 1-2 day old postnatal rats and cultured for a period of up to 13 days. Electrophysiological recordings were conducted using the patch-clamp technique on days 4 to 11, and 13 in vitro (DIV). Key parameters measured included resting membrane potential, input resistance, and cell capacitance.

Notably, the input resistance started to decrease significantly after 6 DIV, whereas the resting membrane potential and cell capacitance remained relatively constant over time. These findings suggest that these neurons undergo differentiation without a concomitant increase in size and provide valuable insights into the electrophysiological behavior and differentiation patterns of neonatal rat hippocampal neurons in a controlled environment.

This research contributes to the broader understanding of neuronal development and has potential implications for neurological studies.

Key words: patch-clamp; excitability; action potential; input resistance; hippocampal neuron.

Introduction

In vitro studies of hippocampal neurons derived from neonatal rats have been instrumental in advancing our understanding of neuronal development, intrinsic excitability, and synaptic integration. The hippocampus, a central structure in the mammalian brain, plays a critical role in learning, memory formation, and spatial navigation. Within this region, CA1 pyramidal neurons are particularly well-characterized for their distinct electrophysiological profiles and developmental trajectories (Kandel et al., 2013; Spruston and Johnston, 1992).

Culturing hippocampal neurons provides a controlled environment to investigate how intrinsic membrane properties evolve during early differentiation. Parameters such as resting membrane potential, input resistance, and membrane capacitance are key indicators of neuronal maturity and functional readiness. These properties are shaped by the dynamic expression of ion channels, changes in membrane morphology, and the establishment of synaptic networks (Debanne et al., 2019; Bean, 2007).

This study focuses on CA1 neurons cultured from neonatal rats, examining their electrophysiological characteristics between 4 and 13 days in vitro (DIV) – a critical window during which neurons undergo rapid differentiation and begin to exhibit mature firing patterns. By tracking changes in resting membrane potential, input resistance, and capacitance, we aim to elucidate the timeline and mechanisms of neuronal maturation in vitro.

Materials and methods

Primary cultured hippocampal neurons were extracted from neonatal rats at 1-2 postnatal day. The neurons selected for electrophysiological recordings exhibited prominent pyramidal morphology with well-defined dendritic processes. The cultures were sustained for a period of 13 days. A recovery post-isolation period was obligatory, as no detectable calcium currents were observed prior to 4 days in vitro (DIV). Electrophysiological experiments were conducted on 4 DIV, 5 DIV, 6 DIV, 7 DIV, 8 DIV, 9 DIV, 10 DIV, 11 DIV, and 13 DIV.