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Īslaicīga pastāvīgā magnētiskā lauka ietekmes uz laboratorijas dzīvnieku fizioloģiskām funkcijām atkarībā no indukcijas vektora virziena

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Saturs

1. Anotācija

Arvien plašāk sadzīvē ienāk pastāvīgie magnēti – ar tiem saskaramies skaļruņos, ūdens attīrīšanas ierīcēs, visai plašs ir magnētisko aproču, auskaru un citu "ārstniecisko" izstrādājumu klāsts. Medicīnas praksē pastāvīgu magnētisko lauku (PML) izmanto ārstniecībā. Literatūrā sastopama informācija par PML izmantošanu epilepsijas ārstēšanā, brūču un kaulu lūzumu ātrākai sadzīšanai.

Ir izvirzīta hipotēze, ka viens no PML parametriem, no kura atkarīga gan lauka efektivitāte, gan fizioloģiskās reakcijas kvalitatīvās izpausmes, ir magnētiskā lauka vektora orientācija pret dzīvnieka ķermeņa un smadzeņu anatomiskām struktūrām.

Elektrofizioloģiskos un etoloģiskos eksperimentos tika izmantoti: "Wistar" populācijas žurku tēviņi (Rattus norvegicus), nelīnijas lauku peļu (Lasiopodomys brandtii) tēviņi un Šinšillas trušu (Oryctolagus cuniculus) tēviņi. Dzīvnieku anestēzijā izmantoja uretānu, ksilazīna un ketamīna maisījumu un nembutālu. Eksperimentos tika izmantoti samārija un kobalta sakausējuma magnēti; to izmērs bija 20x20x10 mm, inducētā magnētiskā lauka intensitāte 1mm attālumā no virsmas ir 250 mT. Trušu elektroencefalogrammas un žurku elektrogrammu reģistrācijai izmantoja biosignālu pastiprinātāju.

Mākslīgs vidējas intensitātes īslaicīgi aplicēts pastāvīgais magnētiskais lauks (viīPML), kas aplicēts laboratorijas dzīvnieka ķermenim vai galvai, izraisa plaša spektra tūlītējas funkcionālas izmaiņas visā organismā, t.sk., ietekmē monoamīnu koncentrāciju smadzenēs, smadzeņu vadības un psihiskās funkcijas, veģetatīvo funkciju regulācijas mehānismus un dzīvnieku uzvedību. Tika apstiprināta hipotēze, ka būtisks viīPML parametrs, no kura atkarīga gan lauka efektivitāte, gan fizioloģiskās reakcijas kvalitatīvās izpausmes, ir magnētiskā lauka vektora orientācija pret dzīvnieka ķermeņa un smadzeņu anatomiskām struktūrām. Viena no viīPML ietekmes uz smadzenēm izpausmēm ir psihosomatisko procesu kavēšana, par ko liecina somatosensoro izsaukto potenciālu latento periodu pagarināšanās, elektrokortikogrammas zemas frekvences viļņu amplitūdas palielināšanās, instinktīvo uzvedības reakciju gausināšanās.

2. Abstracts

Static magnetic fields are of frequent occurrence on the Earth. The Earth by itself is a forceful magnet, which has influence on all processes happening on it. In daily needs static magnets become more and more wide-spread – they can be found in loud-speakers, water-purification systems, very popular are magnetic bracelets, earrings and other "medicative" ware exposure. In medicine praxis static magnetic field (SMF) is used in cure. In literature it is possible to find information about SMF use in epilepsy treatment, for faster healing of injuries and bone fractures.

Author's hypothesis is that important mean intensity short-time applied static magnetic field (misSMF) parameter, on which depends field efficiency as well as physiological reaction qualitative utterance, is the magnetic field vector orientation against animal's body and brain anatomical structures.

In electrophysiological and ethological experiments "Wistar" population rat males (Rattus norvegicus), outbreed field mouse (Lasiopodomys brandtii) males and Chinchilla rabbit (Oryctolagus cuniculus) males were used. For anaesthesia purpose urethane and ketamine in combination with xylazine and also nembutal were used. Biophysical amplifier was used for electrogram registration.

Artificial medium intensity short-term static magnetic field misSMF applied onto the body or head of a laboratory animal evokes instant functional changes of wide spectrum in the whole organism, int.al., influences monoamine concentration in the brain, brain control and mental functions, regulation mechanisms of vegetative functions and animal behaviour. Hypothesis is confirmed: significant misSMF parameter on which both field efficiency and qualitative expressions of physiological reactions depend is magnetic field vector orientation against animal body and brain anatomical structures. One of the expressions of misSMF influence on the brain is the retardation of psychosomatic processes, testified by the extension of the latent periods of somatosensory potentials, increase of low frequency wave range of electrocorticograms, slowing down of instinctive behaviour reactions.

3. Аннотация

Постоянные магниты все прочнее занимают место в нашей жизни – с ними встречаемся в аудио системах, в устройствах по очистке воды и все чаще в предметах «медицинского» назначения. Медицина давно нашла применение постоянному магнитному полю (ПМП). Все чаще встречаются статьи по использованию ПМП для лечения эпилепсии, сращения переломов и заживления ран.

Выдвинута гипотеза, что одним из важных параметров ПМП, от которого зависит эффективность воздействия поля, является ориентация ПМП относительно тела и структур головного мозга животного.

В электрофизиологических и этологических экспериментах использовались: крысы “Wistar” (Rattus norvegicus), полёвки (Lasiopodomys brandtii) и кролики Шиншиллы (Oryctolagus cuniculus). Для наркоза использовался уретан, смесь ксилазина и кетамина, а также нембутал. Различные конфигурации ПМП генерировались магнитами из сплава самария-кобальта, размеры 20x20x10 mm, с интенсивностью поля на расстоянии 1mm от поверхности магнита 250 mT. Для записи биоэлектрической активности использовали усилитель биосигналов.

Воздействие искусственным средней интенсивности кратковременным постоянным магнитным полем (сикПМП) на голову или тело животного, вызывает функциональные изменения широкого спектра во всем организме, в том числе изменения концентрации моноаминов в головном мозге, функции управления, изменяется регуляторная вегетотативная функция и поведение животных. Подтвердилась гипотеза о том, что важным параметром сикПМП, от которого зависит эффективность воздействия поля и качественное проявление физиологических реакций, является ориентация ПМП относительно тела и анатомических структур головного мозга животного. Воздействие сикПМП на животное ярко проявляется через торможение психосоматических процессов, что выражено в увеличении латентного периода вызванных потенциалов, усилении амплитуды низкочастотных волн в электрокортикограмме, а также торможении инстинктивных поведенческих реакций.

Izmantotie apzīmējumi

ML – magnētiskais lauks,

PML – pastāvīgais magnētiskais lauks,

viīPML – vidējas intensitātes īslaicīgi aplicēts pastāvīgais magnētiskais lauks.

DA – dopamīns,

DOPAC – dihidroksifenīlacētāta skābe,

NA – noradrenalīns,

5-HT – 5-hidroksitriptamīns jeb serotonīns,

5-HIAA – 5-hidroksiindoletiķskābes.

Apzīmējumi magnētu novietojumam (resp., viīPML orientācijai) pret dzīvnieka galvu:

lS-kN - pie labās puslodes (l) – dienvidu pols (S), pie kreisās puslodes (k) – ziemeļu pols (N);

lN-kS - pie labās puslodes (l) – ziemeļu pols (N), pie kreisās puslodes (k) – dienvidu pols (S);

lN-kN – pie labās puslodes (l) – ziemeļu pols (N), pie kreisās puslodes (k) – ziemeļu pols (N);

lS-kS – pie labās puslodes (l) – dienvidu pols (S), pie kreisās puslodes (k) – dienvidu pols (S);

4. Pētāmās problēmas nostādne un aktualitāte

Pastāvīga magnētiskā lauka (PML) iespējamo ietekmju uz dzīvajiem organismiem izpēte ir nozīmīga vairākos aspektos.

Vispirms, vispārbioloģiska interese ir par dabīgas izcelsmes PML, proti, Zemes magnētiskā lauka mijiedarbību ar dzīvo dabu – augiem, mikroorganismiem, dzīvniekiem un cilvēkiem. Šajā virzienā būtiska ir magnētiskā lauka kā telpiskās orientācijas signāla uztveres un izmantošanas mehānismu izpēte.

Otrkārt, patstāvīgu interesi rada mākslīgi inducēta vāja PML ietekmes uz cilvēka (dzīvnieku) organismu izpēte, jo ārstniecībā un dziedniecībā diezgan plaši tiek pielietota šādu lauku iedarbība, kaut arī iegūtajam ārstnieciskajām efektam trūkst korekts zinātnisks pamatojums.

Treškārt, pamazām attīstās un apstiprina savu lietderību pētījumi, kuros PML tiek izmantots kā rīks biofizikālo likumsakarību izzināšanai molekulārā un zemmolekulārā līmenī.

Katrā no šiem virzieniem pēdējā desmitgadē gūti nozīmīgi atklājumi, kas ļāvuši izvirzīt principiāli jaunas darba hipotēzes turpmākiem pētījumiem. Piemēram, pētījumi, kuru mērķis bija noskaidrot PML antihipertensīvā efekta mehānismus, ne tikai precizēja priekšstatus par PML ietekmi uz vazodilatācijas signālmolekulu (NO) veidošanos asinsvadu sieniņā un kalcija kanālu vadāmību gludās muskulatūras šūnās, bet ļāva arī pamatot oriģinālu hipotēzi par iespējamu PML modulācijas mehānismu dzīvnieka organismā, kurā kā modulējošais faktors darbojas sirdsdarbības frekvence (Okano, Ohkubo, 2005).

Klīniskās fizioloģijas jomā pēdējā desmitgadē iezīmējusies pāreja no magnetoterapijas empīrisku rezultātu uzkrāšanas uz PML iedarbības mehānismu izpēti. Būtiskākie rezultāti iegūti pētījumos par PML stimulējošo ietekmi uz kaula reģenerāciju, angioģenēzi, mikrocirkulāciju (Basford, 2001), spēju mazināt nociceptīvu signālu izraisītās sāpju sajūtas (Segal et al., 2001).

Magnetorecepcijas jomā noskaidrota intracellulāro magnetjūtīgo molekulāro veidojumu fizikālā daba, un turpina papildināties neirofiziologu priekšstati par tām mugurkaulnieku smadzeņu darbības īpatnībām, uz kurām balstās magnetoreceptīvo orientācijas reakciju vadība (Cain S., et al., 2005). Situāciju pētījumu jomā, kurā iekļaujas promocijas darbs, var raksturot kā pāreju uz PML neirofizioloģisko efektu padziļinātu izpēti. Uzkrāto fenomenoloģisko faktu kopums kā aktuālu izvirza vairāku nozīmīgu apgalvojumu eksperimentālu pārbaudi.

Cilvēka (dzīvnieka) smadzenes ir jutīgas pret vājas intensitātes PML; spilgts tam apliecinājums ir objektīvi konstatējamās izmaiņas smadzeņu darbībā ģeomagnētiskās aktivitātes maiņu laikā, kad lauka intensitātes svārstības iekļaujas pikoteslu diapazonā (McLean et al., 2001).

PML iedarbība uz organismu izraisa organisma (šūnas, bioloģiskā procesa) tūlītēju – īslaicīgu un atgriezenisku – atbildes reakciju, kuras izpausmes (gadījumā, ja lauka iedarbība ir ilgstoša jeb vairākkārtēja) var uzkrāties un veidot noturīgas morfofunkcionālas izmaiņas.

Eksperimentu ar laboratorijas dzīvniekiem rezultāti demonstrē PML īslaicīgas ietekmes uz smadzenēm izpausmju daudzpusību. Parādīts, ka 50 minūšu ilga 30 mT intensitātes PML iedarbība izraisa destruktīvas izmaiņas jūras cūciņu vairākos smadzeņu apvidos – hipotalāmā, lielo puslodžu garozā, smadzenītēs (Bregadze, 1988). Arī truša smadzenēs vāja (20 – 30 mT) un īslaicīga (3 min) PML iedarbība spēj izraisīt neiroglijas strukturālas izmaiņas, kuras turpinās vēl vairākas dienas pēc PML aplikācijas (Холодов, 1982).

Pastāvīgā magnētiskā lauka un dzīvā organisma mijiedarbībā magnētiskais lauks iesaistās kā multiparametrisks faktors, proti, nozīmīga ir ne tikai lauka intensitāte un iedarbības ilgums (arī periodiskums), bet arī lauka frekvence (ja bioloģiskajā struktūrā notikusi ārēja pastāvīga magnētiskā lauka endogēna pārveide par mainīgu) un lauka orientācija pret bioloģisko substrātu.

5. Hipotēze

Viens no PML parametriem, no kura atkarīga gan lauka efektivitāte, gan fizioloģiskās reakcijas kvalitatīvās izpausmes, ir magnētiskā lauka vektora orientācija pret dzīvnieka ķermeņa un smadzeņu anatomiskām struktūrām.

6. Darba mērķis un uzdevumi

Mērķis:

noskaidrot mākslīga vidējas intensitātes (līdz 250mT) pastāvīga magnētiska lauka īslaicīgās (līdz 15 minūtēm) ietekmes tūlītējās izpausmes laboratorijas dzīvnieku (trušu, peļu, žurku) smadzenēs un organismā kopumā.

Uzdevumi:

- noskaidrot monoamīnu koncentrācijas izmaiņas žurkas smadzeņu audos *vidējas intensitātes īslaicīga pastāvīga magnētiskā lauka* (viīPML) ietekmē atkarībā no lauka vektora orientācijas pret smadzenēm;
- noskaidrot viīPML indukcijas vektora orientācijas ietekmi uz laboratorijas dzīvnieku (trušu un žurku) lielo puslodu garozas bioelektrisko aktivitāti;
- noskaidrot viīPML ietekmju uz galvas smadzeņu sirdsdarbības vadības centriem atkarību no indukcijas vektora orientācijas attiecībā pret dzīvnieka galvas anatomiskajām struktūrām;
- noskaidrot viīPML vektora orientācijas ietekmi uz laboratorijas dzīvnieku instinktīvo uzvedību, izmantojot atvērtā lauka metodi.

7. Novitāte

Būtisks viīPML parametrs, no kura atkarīga gan lauka efektivitāte, gan fizioloģiskās reakcijas kvalitatīvās izpausmes, ir magnētiskā lauka vektora orientācija pret dzīvnieka ķermeņa un smadzeņu anatomiskām struktūrām.

Pirmo reizi izdevies parādīt smadzeņu audu īpašu jūtību pret viīPML, ja abpus laboratorijas dzīvnieku galvai novietoti magnētu viennosaukuma poli. Noskaidrots, ka laboratorijas dzīvniekiem (žurkām) raksturīga individuāli atšķirīga jūtība pret viīPML.

8. Autora ieguldījums, darba aprobācija un publikāciju saraksts

Autors patstāvīgi veicis un vadījis visus eksperimentus, izņemot elektrofizioloģisko eksperimentu ar trušiem (eksperimenta vadītājs Dr. med. P.Gustsons) un monoamīnu koncentrāciju noteikšanu izolētos smadzeņu audos (eksperimenta vadītājs Dr. biol. Š. Svirskis). Autors patstāvīgi veicis rezultātu statistikas apstrādi un iegūto rezultātu interpretāciju.

Eksperimentos un datu apstrādē līdzdarbojās arī studenti, apkopojot savu veikumu bakalaura un maģistra darbos.

Par darba rezultātiem ziņots četrās **starptautiskās konferencēs**:

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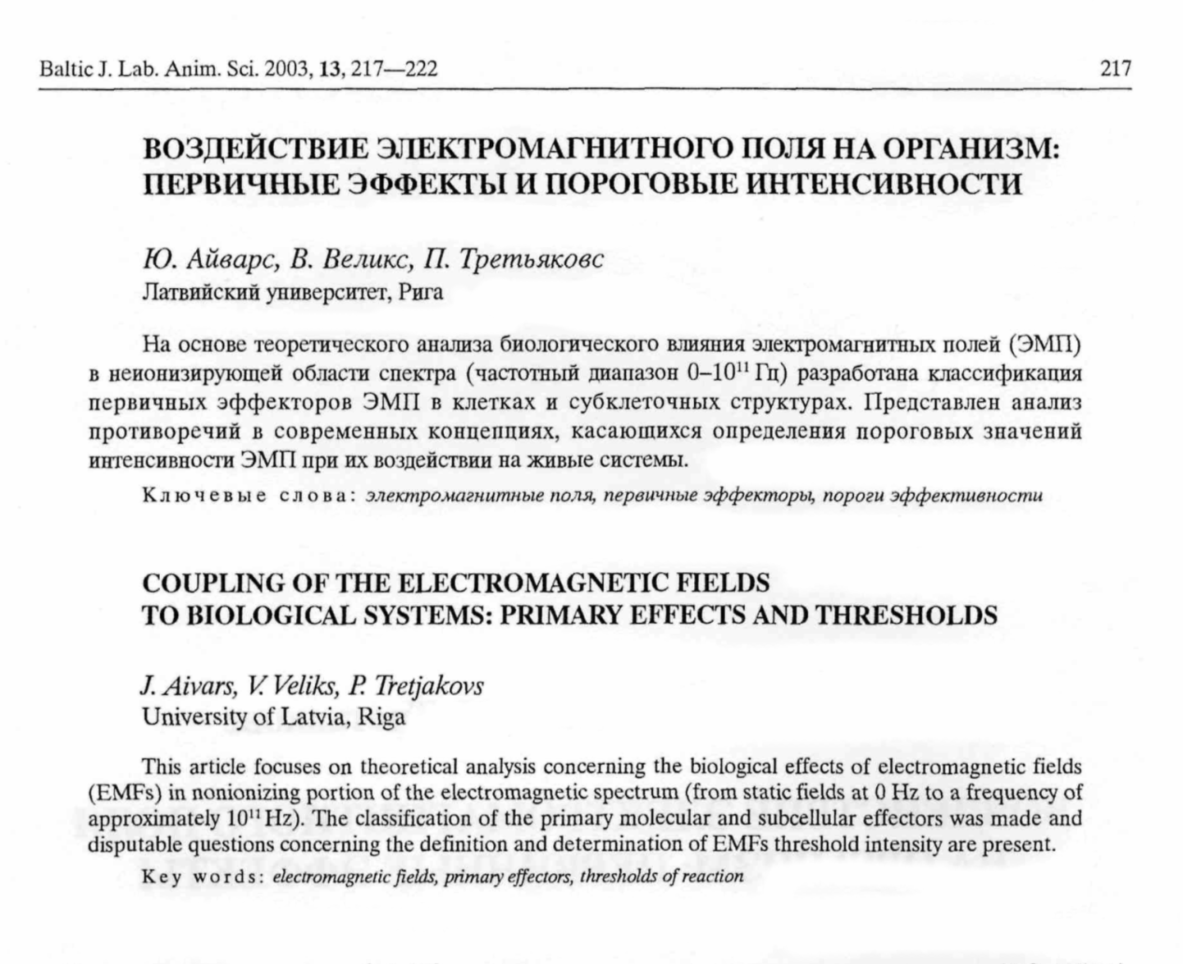
9. Rezultāti

9.1. Elektromagnētiska lauka ietekme uz organismu: primārie efekti un sliekšņu intensitātes



Baltic J. Lab. Anim. Sci. 2003, **13**, 217—222 217

ВОЗДЕЙСТВИЕ ЭЛЕКТРОМАГНИТНОГО ПОЛЯ НА ОРГАНИЗМ: ПЕРВИЧНЫЕ ЭФФЕКТЫ И ПОРОГОВЫЕ ИНТЕНСИВНОСТИ

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На основе теоретического анализа биологического влияния электромагнитных полей (ЭМП) в неионизирующей области спектра (частотный диапазон 0–10^{11} Гц) разработана классификация первичных эффекторов ЭМП в клетках и субклеточных структурах. Представлен анализ противоречий в современных концепциях, касающихся определения пороговых значений интенсивности ЭМП при их воздействии на живые системы.

Ключевые слова: *электромагнитные поля, первичные эффекторы, пороги эффективности*

COUPLING OF THE ELECTROMAGNETIC FIELDS TO BIOLOGICAL SYSTEMS: PRIMARY EFFECTS AND THRESHOLDS

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This article focuses on theoretical analysis concerning the biological effects of electromagnetic fields (EMFs) in nonionizing portion of the electromagnetic spectrum (from static fields at 0 Hz to a frequency of approximately 10^{11} Hz). The classification of the primary molecular and subcellular effectors was made and disputable questions concerning the definition and determination of EMFs threshold intensity are present.

Key words: *electromagnetic fields, primary effectors, thresholds of reaction*

Электромагнитные поля (ЭМП), пронизывающие среду обитания живых существ на Земле, имеют двоякое происхождение:

1) естественное:

- поля и излучения, приходящее из космоса;
- магнитное поле Земли;
- поля, генерируемые живой природой (организмами, клетками, молекулярными структурами);

2) искусственное:

- токи, поля и излучение, генерируемые техническими устройствами, изготовленными человеком.

В данном исследовании анализируются эффекты лишь тех ЭМП, которые являются неионизирующими, т. е. имеют частоту меньшую чем частота инфакрасных лучей (<10^{11} Гц):

- радиочастотные поля (10^4–10^{11} Гц);
- в том числе, микроволновые поля (10^9–10^{11} Гц);
- экстремально низкочастотные поля (10^1–10^3 Гц);
- постоянные ЭМП.

Основным естественным источником всеобъемлющего статического магнитного поля является магнитное поле Земли (МПЗ; геомагнитное поле). Вертикальная составляющая МПЗ наиболее интенсивна в областях магнитных полюсов Земли (около 70 μT), горизонтальная – максимальна в экваториальной зоне (около 30 μT). Активность Солнца также порождает магнитное поле, достигающее Земли, однако даже во время так называемых магнитных бурь интенсивность этого поля не превышает 0,5 μT. Интенсивность статического электрического поля у поверхности Земли составляет около 100 В/м.

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Переменные поля естественного происхождения значительно слабее искусственных полей, генерируемых техническими устройствами. Последние могут иметь исключительно высокую интенсивность. Например, в теле человека, остановившегося под линией высокого напряжения, интенсивность ЭМП может достигать 0,1 мТ [6].

Для защиты человека и живой природы от воздействия ЭМП во многих странах разработаны стандарты предельных значений интенсивностей ЭМП различной частоты [4, 5, 29]. Создатели этих стандартов, как правило, руководствовались предположением, что поля, интенсивность которых ниже определенного минимума, не оказывают какого-либо ощутимого влияния на живые организмы или клетки. Тем не менее, мы еще далеки от четкого представления о физических и биохимических механизмах взаимодействия ЭМП с живыми структурами; в этом направлении ведутся интенсивные разработки.

Цель данного аналитического исследования – выявление биологических эффектов и принципиальных механизмов ЭМП очень низкой интенсивности, а также оценка научной (теоретической) обоснованности концепции порога относительной эффективности влияния ЭМП в различных частотных диапазонах и при различных способах аппликации на основе данных, полученных в последние годы в области физики, электротехники, биологии и медицины.

В список литературы включены лишь новейшие публикации, содержащие веские аргументы по обсуждаемым вопросам.

ПЕРВИЧНЫЕ ЭФФЕКТЫ БИОЛОГИЧЕСКОГО ДЕЙСТВИЯ ЭМП

При анализе эффективности ЭМП важно выявить первичные процессы взаимодействия ЭМП с живыми организмами. Поэтому целесообразно ввести такое понятие, как *первичный эффектор.*

Первичный эффектор – молекулярная структура, представляющая собой биологически значимый функциональный элемент, которая, абсорбируя ЭМП, меняет свои параметры и/или свойства.

Разнообразие физических параметров ЭМП позволяет *a priori* прогнозировать наличие в живых клетках нескольких типов первичных эффекторов. Данный перечень составлен на основе экспериментальных работ, теоретических разработок и обзоров [2, 19, 21, 23, 31, 32].

Первичные эффекторы магнитного поля и первичные проявления:

– свободные радикалы – их функциональные изменения [8, 32];

– магнетиты – изменение их пространственной ориентации [10, 13, 14];

– диамагнитные домены протеинов в биологических мембранах – искажения переноса веществ через мембраны [24, 25];

– магнитосомы – смещение или ротация магнитосом и, как следствие, образование в биологических мембранах пор или каналов [13];

– магнитосенситивные химические реакции – возникновение биологически активных реагентов [32];

– металлопротеиды – искажения их специфических функций [2].

Первичные эффекторы электрического поля и первичные проявления:

– потенциал-зависимые каналы в клеточных мембранах – изменения мембранного потенциала и трансмембранного переноса ионов [28];

– молекулярные рецепторы сигналов в мембранах или цитоплазме клетки – изменения чувствительности к тем или иным сигналам [18];

– агрегаты липидных молекул в биомембранах – изменения проницаемости мембран для жирорастворимых веществ;

– ферменты, фиксированные в мембранах – изменения конформации и ферментативной активности [12, 32];

– гидрофильные поры (каналы) – изменения проницаемости биологических мембран;

– носитель электрических зарядов в межклеточной среде – перемещение носителей зарядов, т. е. возникновение локальных экстрацеллюлярных электрических токов;

– ионы, адсорбированные на клеточной мембране – изменения мембранного потенциала и адгезии клеток [22];

– молекулярные комплексы ДНК – мутагенный эффект [11, 15];

– щелевые межклеточные соединения – изменения проницаемости совмещенных каналов [16].

Новейшие представления в области клеточной физиологии и нейрофизиологии позволяют выявить еще ряд первичных эффекторов и первичных механизмов воздействия ЭМП, оценка значимости которых нуждается, на наш взгляд, в несколько более подробных комментариях.

Взаимодействие внешних ЭМП с электрическими и электромагнитными явлениями эндогенного (биогенного) происхождения (биогенные электрические феномены):

– мембранный потенциал;

– градуальные изменения мембранного потенциала;

– импульсные изменения мембранного потенциала (потенциалы действия);

– циркулярные трансмембранные электрические токи, являющиеся основой бездекрементного распространения потенциалов действия;

– неустойчивые разности электрических потенциалов, возникающие между участками тканей (например, электрические диполи в миокарде и мозге);

– клеточные поверхностные потенциалы;

– трансмуральные разности потенциалов (кожа, стенки сосудов и т. п.).

Взаимодействие ЭМП с механизмами экстрасинаптических межклеточных коммуникаций [26, 33]:

– межклеточные кальциевые волны;

– изменения концентрации ионов в межклеточной среде.

Отдельного упоминания заслуживают механизмы, в которых ЭМП выступает в роли своеобразного ложного сигнала. Речь идет об особом варианте вмешательства ЭМП в механизмы восприятия клеткой специфических сигналов. Можно представить следующую последовательность событий:

1) рецепторный комплекс в мембране клетки или в ядре, предназначенный для определенного сигнала (медиатора, гормона), поглощает ЭМП;

2) абсорбированная порция энергии вызывает конформационные изменения в рецепторе аналогичные тем, которые возникают при взаимодействии рецептора с адекватным ему сигналом;

3) запускается каскад вторичных (внутриклеточных) посредников, что инициирует изменения функционального состояния клетки, характерные для типичной ответной реакции на специфический сигнал.

В этом случае ЭМП будет имитировать эффект сигнала абсолютно другой модальности [27].

ПОРОГОВЫЕ ЗНАЧЕНИЯ ПАРАМЕТРОВ ЭМП

КОНЦЕПЦИЯ ПОРОГА

Данная концепция основывается на представлениях, согласно которым:

1) тот или иной фактор (например, ЭМП) в некотором диапазоне слабых интенсивностей является абсолютно индифферентным для клетки;

2) имеется некоторый характерный пороговый уровень интенсивности минимально достаточной для вызова биологического эффекта;

3) все надпороговые интенсивности, естественно, также являются эффективными.

Вопрос о применимости представлений о порогах относительно электрических и магнитных воздействий актуален именно с точки зрения теоретического обоснования стандартов безвредных уровней интенсивности внешних полей.

ГИПОТЕЗА ТЕПЛОВОГО ШУМА

Эта гипотеза основывается на следующих представлениях: воздействие ЭМП может стать биологически значимым только в том случае, если количество энергии, абсорбированное прямым эффектором (см. выше) превышает количество тепловой энергии, аккумулированной в молекулярной структуре эффектора, т. е.

превышает по интенсивности уровень кинетической энергии хаотического движения молекул и атомов первичной эффекторной структуры.

Новейшие представления биоинформатики позволяют выявить ряд аргументов, ограничивающих применение критерия теплового шума в качестве универсального предельного минимума при определении пороговой интенсивности ЭМП достаточной для вызова биологического эффекта. Этот критерий, несомненно, применим в тех случаях, когда эффект объясняется непосредственно энергетическим воздействием ЭМП на биологическую структуру – мембрану клетки, молекулу фермента и т. п. Однако восприятие ЭМП в качестве информативных сигналов подчиняется качественно иным закономерностям. Речь идет о тех случаях, когда в качестве первичного эффектора выступает какой-либо элемент каскада внутриклеточных вторичных посредников (см. выше – перечень первичных эффекторов). Одной из функций этих каскадов является, как известно, усиление первичного сигнала самой клеткой. Показательны, например, результаты исследований восприятия сверхслабых электромагнитных лучей нейронами эпифиза, которые содержат микрокристаллы кальция, обладающие пьезоэлектрическими свойствами. Выявлена способность этих клеток осуществлять нетермальную идентификацию ЭМП в микроволновом диапазоне [3, 9].

МЕТОДОЛОГИЧЕСКИЕ АСПЕКТЫ

Существует целый ряд объективных обстоятельств, осложняющих определение пороговых значений интенсивности ЭМП минимально достаточных для вызова биологического эффекта:

– различная чувствительность первичных эффекторов к ЭМП;

– зависимость физического механизма (и, тем самым, эффективности) воздействия ЭМП от значений его различных параметров ЭМП (частота, структура поля, временные модуляции параметров поля); различные значения параметров и разнообразные их комбинации могут вызывать качественно различные эффекты;

– комплексный характер воздействия ЭМП на биологический объект, включающий как термальные, так и нетепловые механизмы;

– накопление первичных и вторичных эффектов ЭМП, включая пространственную суммацию и суммацию во времени;

– зависимость пороговой интенсивности ЭМП от функционального состояния эффекторных клеток; показано, что клетки в условиях стресса более чувствительны к влиянию различных ЭМП в связи с накоплением в них особых стресс-протеинов [7, 30].

В таблице представлена попытка классификации биологических эффектов ЭМП, подчеркивающая их разнообразие, которое само по себе

Таблица/Table

Классификация биологических эффектов ЭМП
Classification of biological effects of EMFs

I. *Незамедлительные эффекты* (обусловленные изменениями в структуре первичного эффектора):
- изменения, вызванные интенсификацией теплового движения (термальный эффект);
- изменения конформации эффекторных молекул, подвижности ионов, конфигурации электронных орбит и т. п. [20];
- взаимодействие внешних ЭМП с эндогенными полями.

II. *Запоздалые эффекты*:
- обусловленные накоплением первичного эффекта;
- обусловленные медлительностью пораженных биологических (физиологических) процессов, например, дефекты хромосом, проявления которых определяются спецификой и биологической ролью определенных генных продуктов [17].

существенно затрудняет создание физически корректной дефиниции и практическое определение порогов интенсивности ЭМП.

ЗАКЛЮЧЕНИЕ

1. Специфика молекулярных структур, абсорбирующих энергию неионизирующих электромагнитных полей и излучения, зависит от спектрального состава и интенсивности ЭМП, условий экспозиции и функционального состояния живой структуры.

2. В пределах даже одной клетки ЭМП абсорбируется несколькими (многими) первичными эффекторами.

3. Первичные механизмы воздействия ЭМП подразделяются на три основных типа: термальное, нетермальное энергетическое и информативное (сигнальное) воздействие.

4. Чувствительность живых тканей (клеток, организмов) к ЭМП и эффективность ЭМП зависят от функционального состояния биологических структур, абсорбирующих ЭМП.

5. Патологические изменения в клетках могут быть дополнительным фактором (своеобразным ко-стрессором), повышающим чувствительность клетки и потенциирующим эффективность ЭМП.

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9.2. Monoamīnu koncentrācijas izmaiņas žurku smadzenēs pēc iedarbības ar pastāvīgo magnētisko lauku

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CHANGES OF MONOAMINE CONCENTRATION IN RAT BRAIN UNDER THE INFLUENCE OF A STATIC MAGNETIC FIELD

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The influence of a static magnetic field (SMF) on rat brain was performed by two bitemporally placed samarium–cobalt fused magnets 20×20×10 mm in size. The SMF was strictly symmetrical with magnetic induction intensity 250 mT on the surface of the magnets. Varying of magnetic poles on both sides of the head changed the direction of the magnetic induction vector. Tissue samples of different brain areas—frontal cortex, corpus striatum, hypothalamus, hippocampus—were examined to determine the concentrations of the following neurotransmitters: dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-metoxy-4-hydroxyphenylacetic acid (HVA), noradrenalin (NA), serotonin (5-HT), and 5-hydroxyindolaceatic acid (5-HIAA). The results suggested that the static magnetic field had an influence on brain monoamine metabolism; the effectiveness of SMF application differed to various areas of brain and depended upon the direction of the magnetic field vector relative to the anatomic projection of brain structures.

Key words: *rat brain, static magnetic field, monoamine concentration.*

INTRODUCTION

It has already been established that the functional state of the central nervous system changes under the influence of magnetic fields. This effect has been demonstrated on both systemic and cellular levels of the information processing in the central nervous system (Young, 1969; Холодов, 1975; Холодов и Шишло, 1979; Liburdy and Tenforde, 1986; Gustson *et al.*, 1982; Gustsons, 1992; Ueno and Iwasaka, 1996). Most important, the selective effect of the two opposite directions of static magnetic field has been previously reported by Gustsons and his collaborators. Their data show that the magnetic field applications have prolonging or inhibitory effects on brain functions, depending on the vector direction (Gustsons *et al.*, 1982; Gustsons, 1992; Veliks *et al.*, 2000) and possible effects of magnetic field on the components of biochemical reaction pathways. This includes the enzyme and substrate conformation change in response to the electrical charges on the aminoacids, and the changes of environmental conditions due to magnetic field application alter biochemical reactions in the brain on both solvent phase level as well as protein-membrane complex level (Schwartz, 1979; Easterly, 1982).

Taking the above into account, the aim of this study was to evaluate the influence of static magnetic field on rat brain monoamine metabolism using bitemporally placed magnets with field intensity 250 mT.

MATERIALS AND METHODS

Animals. The acute experiments were carried out on 30 male Wistar population rats with weight 300–400 g (Breeding House of the Latvian State Pharmaceutical Company "Grindex," Latvia). Rats were taken directly from vivaria to the place of experiment; they were kept under standard conditions, and food ("Grindex," Latvia) and water was given *ad libitum*. Rats were anaesthetised by intraperitoneal (i.p.) injection of urethane (770 mg/kg (Shering-Kahlbaum A. G.), and i.p. injection of ketamine (30 mg/kg) mixed with xylasine (3.5 mg/kg) (both DOPHARMA).

Thirty rats were divided into six parts by five animals per group:

1, without anaesthesia and static magnetic field (SMF) influence (C2);

2, with anaesthesia, without SMF influence (C1);

3, with anaesthesia, SMF vector: right side of the head—south pole, left side of the head—North pole (rS-lN);

4, with anesthesia, SMF vector: right side of the head—north pole, left side of the head—South pole (rN-lS);

5, with anesthesia, SMF vector: right side of the head—south pole, left side of the head—South pole (rS-lS);

6, with anesthesia, SMF vector: right side of the head—North pole, left side of the head—North pole (rN-lN).

Experiments were performed according to the guidelines of the European Ethics Committee for Laboratory Animal Science, with the experimental protocol approval by the Latvian LAS Ethics Committee.

Monoamine determination. Concentrations of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-metoxy-4-hydroxyphenylacetic acid (HVA), noradrenaline (NA), 5-hydroxytryptamine or serotin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were determined by using HPLC with electrochemical detection by adapted method from Alburges *et al.* (1993).

Sample preparation. After a 15-minute exposure under the magnetic field, the rats were killed by decapitation. After decapitation, the brains were rapidly removed and dissected on an ice-cooled glass plate. Samples of different brain areas including frontal cortex, striatum, hypothalamus, and hippocampus were isolated, and the tissues were then placed in 1.5 ml Eppendorf micro tubes, frozen on dry ice, weighed and stored at –750 °C until assay. Frozen brain samples were homogenised in 1 ml of 0.05 N perchloric acid solution containing (by weight/volume) 0.064% octanesulphonic acid (OSA), 0.060% heptanesulphonic acid (HSA), 0.004% sodium ethylenediamine tetra acetic acid (EDTA), 0.010% sodium metabisulphite and the whole procedure was carried out on ice. The resulting homogenate was then centrifuged with ventrifugation forces of 30,000 g for 20 min at 40 °C and the supernatant was filtered using 0.45 mm (pore size) HV Millipore filters. The filtrate was injected directly into the liquid chromatography equipment (20 ml).

High performance liquid chromatography (HPLC) analysis. Samples were injected by a Rheodyne 7125 manual sample injection valve onto a 5 mm, ODS (octadecyl silane), 25 cm × 4.6 mm I.D. (inner diameter) (Beckman, USA) reverse-phase column. The mobile phase was 0.02 M citrate/ 0.01 M NaH2PO4, 0.1 mM EDTA, 3 mM OSA, 3 mM HSA, 0.6% (v/v) o-phosphoric acid (85%), 3.5% (v/v) methanol, 7.5% (v/v) acetonitrile. pH was adjusted to 3.0 with diethyl amine. The detector was a Shimadzu L-ECD-6A (electrochemical detector) (Japan) with glassy carbon cell and electrode potential set at 750 mV, with respect to Ag-AgCl reference electrode. Mobile phase flow rate was maintained at 1.1 ml/min using a Waters 510 HPLC pump (USA).

Known standards were analysed in each unknown sample series. Sample values were calculated at peak heights of known standards. All values were in ng/kg of tissue.

Characteristics of a static magnetic field. The SMF was induced by samarium-cobalt fused magnets 20 × 20 × 10 mm in size for impact on the head. The impact of SMF on rat brain was performed by two bitemporaly placed magnets. Magnetic induction intensity on the surface of magnets was 250 mT (Veliks *et al.*, 2000). Variation of magnetic poles at both sides of the head changed the direction of magnetic induction vector. Duration of the impact of SMF on the brain was 15 minutes.

Statistical analysis. Data are expressed as mean ±SD. Differences among groups were analysed using the unpaired Student t-test ($P < 0.05$).

RESULTS

The results demonstrated that there were no statistical differences between the two control groups: with narcosis and without narcosis. The control groups with narcosis had a greater tendency for monoamine concentration.

Table 1 shows monoamine concentration changes in the somatosensory areas of the right and left frontal cortex of brain. The noradrenalin concentration increased in the left frontal cortex under two magnetic vector induction directions: rN-lS and rN-lN.

When magnet induction vector direction in the right cortex was rN-lS and in the left cortex—rN-lS, rS-lS and rN-lN, a statistically significant increase of serotonin (5-HT) concentration was observed.

Interestingly enough, the ratios of 5-hydroxyindolacetic acid/serotonin changed in both brain hemispheres under similar magnetic induction vector directions: rS-lN and rN-lS. This is possible due to changes MAO conformation under the magnetic field.

In Table 2, monoamine changes in the hippocampus of rat brain are demonstrated. Noradrenalin concentration was unchanged in the hippocampus.

The concentration of serotonin (5-HT) statistically decreased under the following magnet induction vector directions: in the right hippocampus N-N; in the left hippocampus S-N and N-N.

Similarly, ratios of the 5-hydroxyindolacetic acid/serotonin changed in both brain hemispheres under magnetic induction vector direction of rN-lN. This may be due to the activation of the serotonin degradation pathway which took place through MAO, in opposite to the somatosensory areas of frontal cortex and hypothalamus.

Table 4 demonstrates changes of the monoamines at the rat hypothalamus. Changes in N-N magnetic field application resulted in a decrease of DOPAC concentration. Serotonin (5-HT) concentration was increased under application of the N-S magnet induction vector direction. The ratio of 5-hydroxyindolacetic acid/noradrenaline decreased in the N-S, S-S, and N-N magnetic induction vector directions.

The serotonin concentration changes were more sensitive to the SMF, and we found that the MF changed the ratio of 5-hydroxyindolacetic acid/noradrenalin, but these changes went in both directions—decrease in the somatosensory ar-

Table 1

MONOAMINE CONCENTRATION CHANGES IN THE SOMATOSENSORY AREAS OF RIGHT AND LEFT FRONTAL CORTEX OF BRAIN

Right frontal cortex	NA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o inf)	624.5±79.94	948.16±215.84	390.0±48.58	0.411
C2 (int, w/o inf)	528.0±115.32	929.67±288.39	357.33±84.21	0.384
rS-IN	628.0±185.62	1128.17±224.99	364.5±74.99	0.323*
rN-1S	713.83±191.65	1166.0±150.05*	413.0±132.89	0.354*
rS-IS	582.5±172.18	1025.17±330.58	355.0±119.77	0.346
rN-1N	705.33±129.82	1094.5±528.24	346.67±57.45	0.317

Left frontal cortex	NA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o inf)	545.5±115.01	967.67±493.51	380.17±102.95	0.393
C2 (int, w/o inf)	539.33±127.02	1066.83±600.26	373.33±99.95	0.35
rS-IN	684.83±267.21	1275.67±456.22*	378.83±76.15	0.297*
rN-1S	714.5±89.95*	1126.83±357.03	379.5±65.66	0.337*
rS-IS	658.33±173.99	1224.67±530.34*	427.0±115.04	0.349
rN-1N	739.5±145.16*	1246.17±656.62*	382.0±57.82	0.307

Abbreviations for group designations and monoamines: C2, without anesthesia and SMF influence; C1, with anesthesia, without SMF influence; rS-IN, the orientation of SMF in the opposite direction; rN-IS, the north pole of the magnet is on the right side of the head and the south pole is on the left side; rS-IS, the south poles are located bitemporally; rN-1N, the north poles are located bitemporally; noradrenalin (NA), serotonin (5-HT) and 5-hydroxyndoleatic acid (5-HIAA).

Values marked with * are significantly different from the control values C1 ($P < 0.05$, t test).

Table 2

MONOAMINE CHANGES AT THE RAT BRAIN HIPPOCAMPUS

Right hippocampus	NA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o inf)	623.33±109.12	451.67±37.04	468.0±50.97	1.036
C2 (int, w/o inf)	622.83±93.60	466.17±42.22	460.67±81.59	0.988
rS-IN	603.83±114.63	432.83±34.80	504.5±94.62	1.166
rN-IS	688.83±83.34	451.0±50.24	520.67±65.89	1.154
rS-IS	670.83±135.08	463.67±59.38	509.67±49.59	1.099
rN-IN	642.0±140.99	403.83±42.25*	526.67±71.68	1.304*

Left hippocampus	NA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o inf)	650.17±113.37	481.67±21.33	491.5±38.82	1.020
C2 (int, w/o inf)	593.83±103.76	443.67±32.54	434.5±80.11	0.979
rS-IN	628.17±122.28	443.83±35.16*	508.17±96.23	1.145
rN-IS	674.33±93.09	468.33±63.40	526.17±56.15	1.123
rS-IS	684.5±168.23	448.67±30.88	534.67±74.16	1.192
rN-IN	637.5±173.30	402.67±54.18*	513.67±52.79	1.276*

For abbreviations see Table 1. Values marked with * are significantly different from the control values C1 ($P < 0.05$, t test).

eas of frontal cortex and hypothalamus, and increase in the hippocampus. It is possible in these cases that the magnetic field changed MAO conformation or utilised 5-HT or 5-HIAA from other cell structures.

DISCUSSION

The mechanisms underlying these and other SMF action phenomenon at present are obscure, because biological effects of static magnetic fields are poorly understood at all levels of organisation—starting from the molecular level and up to the whole organism. It is only possible to speculate upon different mechanisms and to acknowledge the therapeutic or harmful effect caused by short- or long-term exposures to a static magnetic field.

It is unlikely that such somatosensory evoked potential latency prolongation was caused only by changes in conduction velocity in axons. Moreover, experiments on lobster giant nerve showed that SMF (B = 1.2 T) did not change membrane resting potential level, membrane capacity, axoplasmic and external medium resistance and average

Table 3

MONOAMINE CHANGES AT THE STRIATUM

R. Striatum	NA	DA	DOPAC	DOPAC/DA	HVA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o f)	280.83±209.32	9394.33±1379.032	847.50±77.08	0.090	717.83±140.23	452.33±88.42	569.83±106.10	1.260
C2 (int, w/o f)	372.0±151.27	8810.50±1678.04	963.50±153.19	0.109	675.83±218.73	544.50±181.63	607.67±115.72	1.116
rS-IN	252.67±112.55	10756.83±2115.78	858.67±147.0	0.080	728.5±140.68	559.17±122.41	610.50±44.98	1.092
rN-IS	318.83±197.23	10924.83±2056.76	984.67±308.77	0.090	864.83±234.34	569.0±132.15	660.50±119.34	1.161
rS-IS	302.0±136.37	11071.67±1879.76	1083.5±416.05	0.098	871.33±174.11	574.33±95.77*	642.50±68.01	1.119
rN-IN	252.33±81.14	13114.17±1270.49*	1119.50±208.59*	0.085	1013.0±257.97*	545.17±65.87	634.67±50.22	1.164

L. Striatum	NA	DA	DOPAC	DOPAC/DA	HVA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast. w/o f)	369.50±267.39	9274.33±1431.52	1007.83±317.82	0.109	855.33±320.76	580.50±244.23	714.0±258.89	1.230
C2 (int. w/o f)	274.33±111.42	10178.5±928.11	1084.83±262.77	0.107	773.17±163.83	523.50±80.57	641.17±75.55	1.225
rS-IN	295.0±116.60	10441.33±1558.93	846.0±206.91	0.081	730.5±210.85	581.83±118.20	625.0±50.36	1.074
rN-IS	330.83±113.13	10508.5±1754.11	916.66±145.29	0.087	867.83±150.89	600.67±88.38	666.0±85.12	1.109
rS-IS	270.6±127.94	10464.8±1696.78	851.60±231.6	0.081	851.6±315.51	592.20±101.87	619.8±72.45	1.047
rN-IN	194.83±125.83	10847.66±2507.16	953.83±119.83	0.1	781.66±226.56	734.66±422.30	616.00±72.74	1.0

For abbreviations see Table 1. DA, dopamine; DOPAC, 3,4-dihydrophenylacetic acid. Values marked with * are significantly different from the control values C1 ($P < 0.05$, t test).

Table 4

MONOAMINES CHANGES AT THE HYPOTHALAMUS

Hypothalamus	NA	DA	DOPAC	DOPAC/DA	5-HT	5-HIAA	5-HIAA/5-HT
C1 (anast, w/o inf)	2658.33±529.56	409.21±77.28	41.47±12.09	0.101	770.67±140.86	523.55±83.94	0.679
C2 (int, w/o inf)	2713.17±557.41	384.97±120.25	40.92±8.05	0.106	718.33±200.69	508.50±110.51	0.708
rS-IN	2561.17±465.86	460.41±101.19	41.85±8.01	0.091	853.5±153.59	469.33±62.27	0.550
rN-IS	2652.0±284.15	464.01±95.95	41.87±15.3	0.090	923.17±85.0*	470.5±81.72	0.510*
rS-IS	2544.33±481.56	484.10±123.31	43.53±15.57	0.090	836.17±188.75	465.18±117.05	0.556*
rN-IN	2347.33±488.14	395.53±95.59	32.35±11.13*	0.082	807.67±168.77	400.91±116.01	0.496*

For abbreviations see Tables 1 and 2. Values marked with * are significantly different from the control values C1 ($P < 0.05$, t test).

maximum-rate of sodium and potassium conductance. Also, nerve conduction velocity was not changed by impact with SMF (Schwartz, 1978; 1979). However, contradictory findings have also been reported by (Reno, 1969). Thus, we consider that conduction velocity in axons may have some effect, but we rather believe that mainly the strength and functional state of the synaptic transmission had been influenced in the brain.

Another possible mechanism by which the influence of a SMF is realised on the nervous system may be associated with the cell membrane lipid, phospholipids, and protein macromolecular compound sensitivity to SMF (Colbec *et al.*, 1986; Liburdy and Tenforde, 1986; Ueno and Iwasaka, 1996). The cell membrane is considered as a biological structure that may be influenced by electromagnetic and magnetic fields. The existence of a large potential gradient due to asymmetrically charged macromolecules, cell surface change density and ion mobility, and interactions with structured water are believed to render membranes sensitive to electric and magnetic fields. Also, the behaviour of small and mobile signal molecules could be strongly altered in the SMF, thus causing changes in the physiological state of the network of neurons. A good example could be signal molecules NO, ATP, or ADP, the diffusion of which in surrounding tissue have a neuromodulatory effect and probably could be altered by SMF. The movement of charged particles in the blood circulatory system could be significantly altered as well. Most importantly, the blood supply in the brain is not symmetrical, and therefore, may explain some effect by the direction of SMF (Simon, 1992; Ueno and Iwasaka, 1996).

Finally, the SMF action on the brain may be realised by changing the intensity of reactions for synthesis and release of some neurotransmitters (Young, 1969). The molecules of many enzymes have free electrons. These electrons in macromolecules circulate along their own orbit and are a determining factor for the activity of enzymes. When this electron changes its orbit or orbit energy levels, enzyme activity changes as well. The orbit of these free electrons could be altered in SMF. It has been unequivocally shown that acetylcholinesterase under influence of SMF with induction more than 0.27 T is activated approximately up to 40%, and at 1.7 T up to 250% (Easterly, 1982).

Because of the short time of influence (only 15 minutes) we observed small changes in monoamine concentrations. Probably, with an increase in time these changes may be more intensive. In our investigation the serotonin pathway was affected, which indicates the inhibitor effect of SMF in short-term application.

The experimental results demonstrated that:

1. In hypothalamus: (a) the concentration of DOPAC and ratio DOPAC/DA decreased, when at both sides of the head were north poles (rN-lN) or north pole at the right side of the head and south pole at the left side of head (rN-lS); (b) the concentration of 5-HT increased if the magnet poles location was rN-lS; (c) the ratio between HIAA/5-HT decreased in cases of magnet location rN-lS, rN-lN and also if at both sides of the head were placed south poles (rS-lS).

2. In corpus striatum: (a) the concentration of DA and DOPAC in right striatum increased at magnet poles location rN-lN; (b) the concentration of 5-HT in right striatum increased when magnet pole locations was rN-lS, rS-lS and also if at the right side of head was the south pole and at the left side the north pole (rS-lN).

3. In hippocampus: (a) the concentration of 5-HT in both right and left hippocampus decreased at magnet pole location rN-lN; (b) the ratio of HIAA/5-HT increased at left hippocampus at magnet pole location rN-lN.

4. In somatosensory area of the frontal cortex: (a) the concentration of 5-HT in the right cortex increased at magnet pole location rN-lS; (b) the concentration of 5-HT in the left cortex increased in the following magnet pole locations—rS-lN, rN-lN and rS-lS; (c) the ratio of HIAA/5-HT in both right and left cortex decreased at magnet pole location rN-lS, rS-lN; (d) the concentration of NA in left cortex increased at magnet pole locations rN-lS, rN-lN.

The results allow to conclude that the nervous system reacts differently depending on the direction of the magnetic induction vector.

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MONOAMĪNU KONCENTRĀCIJAS IZMAIŅAS ŽURKU SMADZENĒS PĒC IEDARBĪBAS AR PASTĀVĪGO MAGNĒTISKO LAUKU.

Pastāvīgā magnētiskā lauka radīšanai izmantojām samārija un kobalta sakausējuma magnētus (2 × 2 × 1 cm). Magnētiskais lauks bija simetrisks, un magnētiskās indukcijas vektora lielums uz magnētu virsmas bija 250 mT. Mainot magnētisko polu novietojumu abās pusēs žurkas galvai, panākta magnētiskā vektora orientācijas izmaiņas. Izmeklēti audu paraugi no šādiem smadzeņu apvidiem – frontālās garozas, *corpus striatum*, hipotalāma, hipokampa; tajos noteiktas šādu neiroaktīvo vielu koncentrācijas – dopamīna (DA), 3,4-dihidroksifenilacetātskābes (DOPAC), 3-metoksi-4-hidroksifenilacetātskābes (HVA), noradrenalīna (NA), serotonīna (5-HN) un 5-hidroksiindolskābes (5-HIAA). Rezultāti rāda, ka pastāvīgā magnētiskā lauka iedarbība uz smadzenēm izraisa izmaiņas monoamīnu metabolismā, un lauka iedarbība ir atkarīga no smadzeņu struktūru specifiskām īpatnībām un magnētiskā lauka indukcijas vektora virziena attiecībā pret katru anatomisko struktūru.

9.3. Truša smadzeņu bioelektriskās aktivitātes pārmaiņas, iedarbojoties lokāli ar pastāvīgu magnētisko lauku uz amigdalārajiem kodoliem

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RABBIT BRAIN BIOELECTRICAL ACTIVITY: CHANGES UNDER THE IMPACT OF A PERMANENT MAGNETIC FIELD APPLIED LOCALLY ON AMYGDALOID NUCLEI

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The regulation mechanism of the activity of associative neuronal intercommunication links in neuronal nets was studied under the influence of permanent magnetic fields (PMF). Our previous experiments on animals and physiological observations on humans showed that if the head was placed in a PMF, the changes of the functional state of the central nervous system depended on the orientation of the brain in the PMF. This work attempted to arouse changes of bioelectrical activity of brain by a PMF almost locally on the anterior region of the amygdaloid nuclei, separately with the south pole and the north pole of magnet-electrode. For this purpose, a ferromagnetic electrode was introduced in the amygdaloid nuclei. The study was carried out on 17 rabbits in chronic experiments. Each animal remained fully awake and was placed in a special box. If the south pole of the magnetic electrode was located in the amygdaloid nuclei, generalised hypersynhronous convulsive activity arose spontaneously 5–10 minutes after the beginning of electrode magnetisation in all thalamic and cortical structures of brain. Vice versa, when the north pole of the magnet was placed in amygdaloid nuclei, an asynchronous slow activity developed in all neuronal structures with high amplitude potentials (>100 μV), similar to teta and delta waves in man.

Key words: *permanent magnetic field, amygdala, brain bioelectrical activity.*

INTRODUCTION

An important problem in neurophysiology and neurocybernetics is to determine the mechanism regulating the distribution of excitatory and inhibitory links in neuronal nets during information processing. It is known that the electrochemical field of the brain determines the distribution and level of excitatory and inhibitory processes in cerebral neuronal structures. That was demonstrated by the formation of dominant focus in cerebral cortex (Русинов и Густсон, 1968; Русинов, 1969) and in experiments with spreading depression (Густсон, 1963; Mescherskii and Gustson, 1964). These two processes were accompanied by oscillations of direct-current potential of the cerebral cortex. It is known that all electrochemical processes which are associated with ion curent flow generate their own magnetic field (Gardner-Medwin *et al.*, 1991). Magnetic field and ion current are interconnected and are very sensitive to the influence of external magnetic fields (Klitzing, 1991).

It has been demonstrated that the nature of the changes of the nervous system activity under the influence of a permanent magnetic field (PMF) depends on head orientation according to the magnetic field induction vector direction (Густсон, 1987; Gustsons, 1992). This observation led to the development of specialised equipment for the study of the PMF effect (Густсон и др., 1981; Gustson *et al.*, 1982; Густсон и Думеш, 1989; Густсон и др., 1989). In all the above studies, a PMF was applied to the whole brain. Under such conditions, it was not possible to identify the tissue or substance which is more sensitive to PMF action. Therefore, it was decided to apply a PMF almost locally to a neuronal structure of rabbit brain. If the changes in brain bioelectrical activity due to a PMF on the whole head depended on the magnetic field induction vector direction to the sagital plane of the brain (Густсон, 1987; Gustsons, 1992), the nervous system also should react differently to almost local impact with the PMF depending on which magnetic pole (south or north) is used, and a shift of the functional neuronal connections is expected. The nuclear complex of amygdala participates in the generation of hypersynchronic neuronal discharges of brain and stimulates generalised epileptic seizures. Also, the amygdaloid nuclei located in the rostral part of the brain stem are suitable neuronal structures for the study of the PMF influence on the central nervous system, since the neuronal organisation and morphology of cerebral cortex and amygdaloid nuclei are similar (Braak and Braak, 1983; Millhouse and De Olmos, 1983; McDonald, 1984; Carlsen and Heimer, 1988), and since there are extensive widespread links between amygdaloid nuclei cerebral cortex and subcortical structures of brain (Wolf and Butcher, 1982; Aggleton and Mishkin, 1984; Krettek and Price, 1978; Porrino *et al.*, 1981; Sripanidkulchai *et al.*, 1984; Carlsen *et al.*, 1985).

MATERIALS AND METHODS

The studies were carried out on 17 chincihilla male rabbits (mass 2.5–3.5 kg) in chronic experiments. The animals were obtained from the Breeding Facility of the Medical Academy of Latvia. They were housed individually and fed a standard diet. Water was supplied *ad libitum*. The temperature was maintained at 17–21 °C and relative humidity at 40–60 %. Operations on the head were conducted according to the traditional methods of asepsis under nembutal anaesthesia (35–40 µg per kg). After operation, a solution containing 300,000 units of penicillin was injected into the animal abdominal cavity. Electrodes for recording were implanted in the brain at stereotaxic coordinates given in the atlas of Fifková and Maršala (1960). Points at which the head was fixed in the stereotaxic apparatus were anaesthetised by a solution of novocaine.

The electrodes for subcortical recordings were stainless steel wires 50–70 µ in diameter coated with Epoxilit. The electrodes used for cortical recordings were silver wires 250 µ in diameter and were led into the skull as far as the lamina vitrea. In the area amygdalarum anterior (AAA), a ferromagnetic electrode wire (diameter 220 µ, length 45–50 mm, tip diameter 50–70 µ) was introduced. The magnetic electrode wire was produced in the Institute of Physics of the Russian Academy of Sciences in Moscow. After magnetisation, a 8–10 mT magnetic induction intensity was recorded on both tips of electrode at 1.0–2.0 mm from its surface. A magnetic induction of 8–10 mT is the threshold for brain reaction to a magnetic field. Magnetic induction (B) was measured with a universal teslometer (range 0.2 mT to 2 T). The maximal magnetic induction was recorded on both tips of the magnet-electrode. Along the length of the electrode, the magnitude of magnetic induction was negligible at a sub-threshold level. The electrode was magnetised by a permanent magnet (samarium and cobalt alloy) with magnetic induction intensity 0.27 T. It was possible to change the magnetic poles (north to south or *vice versa*) on both magnet-electrode tips during an experiment. In some experiments, to study local effects on AAA with PMF (south pole of magnet-electrode), the whole brain simultaneously was placed in a diffuse magnetic field with magnetic induction intensity 56 mT at the middle of the brain.

The electrodes were fixed to the skull with stiracryl. In all cases, monopolar leads were used, and the indifferent electrode was situated near the tuber occipitalis where the bone is very porous and rich in fluid. Recording of brain bioelectrical activity began on the fourth day after operation. Electrodes located in brain and muscles were connected to amplifiers and a computer for data processing. The observations of all experiments and animals were summed, and the spectral functions of biopotential oscillation frequencies and amplitudes were calculated. During the experimental procedure, the animal was awake and its body was fixed in a special box in a free manner. Under such conditions, the animal was quiet and felt unrestrained. On average, five experiments were carried out on each animal with a 2–3 day interval. If inflammation of the head brain was observed, the experiment series was interrupted.

After completion of the experiments, the animals were sacrificed by interavenous injection of nembutal 80 mg·kg^{-1}; and the brain, after fixation in 10 % formalin for twenty-four hours, was cut with a freezing microtome. Localisations of subcortical electrodes were determined in sections of the brain.

The mean data were processed by Fast Fourier Transformation (FFT) and Spectral Power Density. Statistical significance of the effects was assessed by Student's t-test criteria. Changes were considered significant at the level $P<0.05$.

RESULTS

Initial brain background bioelectrical activity from cortical and subcortical leads did not show any signs of irritation or hypersynchronous potentials.

When the magnet-electrode was magnetised such that the electrode tip in AAA was the south pole of the PMF (B= 8–10 mT), then two forms of generalised hypersynchronised activity were observed corresponding to magnet-electrode tip localisation in AAA. In the recordings of bioelectrical activity from each magnet-electrode, only one form of generalised hypersynchronous potential was registered. In the experiments on ten rabbits, 10 minutes after magnetisation of the electrode inserted in the anterior part of amygdala, synchronised bioelectrical activity appeared as convulsive epileptic discharges, which were propagated directly to the motor cortex bilaterally. Several seconds later, the convulsive discharges were recorded in the nuclei ventralis posterior lateralis (VPL) of the thalamic complex. All hypersynchronic discharges were succeeded by desynchronisation of background activity for 5–10 seconds. During the hypersynchronic discharges, in some cases, moderate contractions of hind leg muscles were observed. In experiments on the other seven rabbits, 10 minutes after magnetisation of the electrode such that the south pole of the magnet-electrode was in the AAA at the first generalised spindle activity, synchronous slow waves (1–2.5 Hz, 60–70 µV) and sharp waves or spikes (see Figure 1B) developed. The bioelectrical activity was disrupted by generalised desynchronisation for 5 seconds. Five minutes later, the amplitudes of spindles and slow waves increased. Random sequence groups of generalised hypersynchronous wave discharges (complex: spike and 3 Hz wave) were superimposed (see Figure 1C) on this background of brain bioelectric activity. That type of discharge is similar to the "petite male" activity of man.

When the whole brain was exposed to a homogeneous PMF with an induction intensity of more than 56 mT at the middle of the brain, after three minutes the bioelectrical activ-

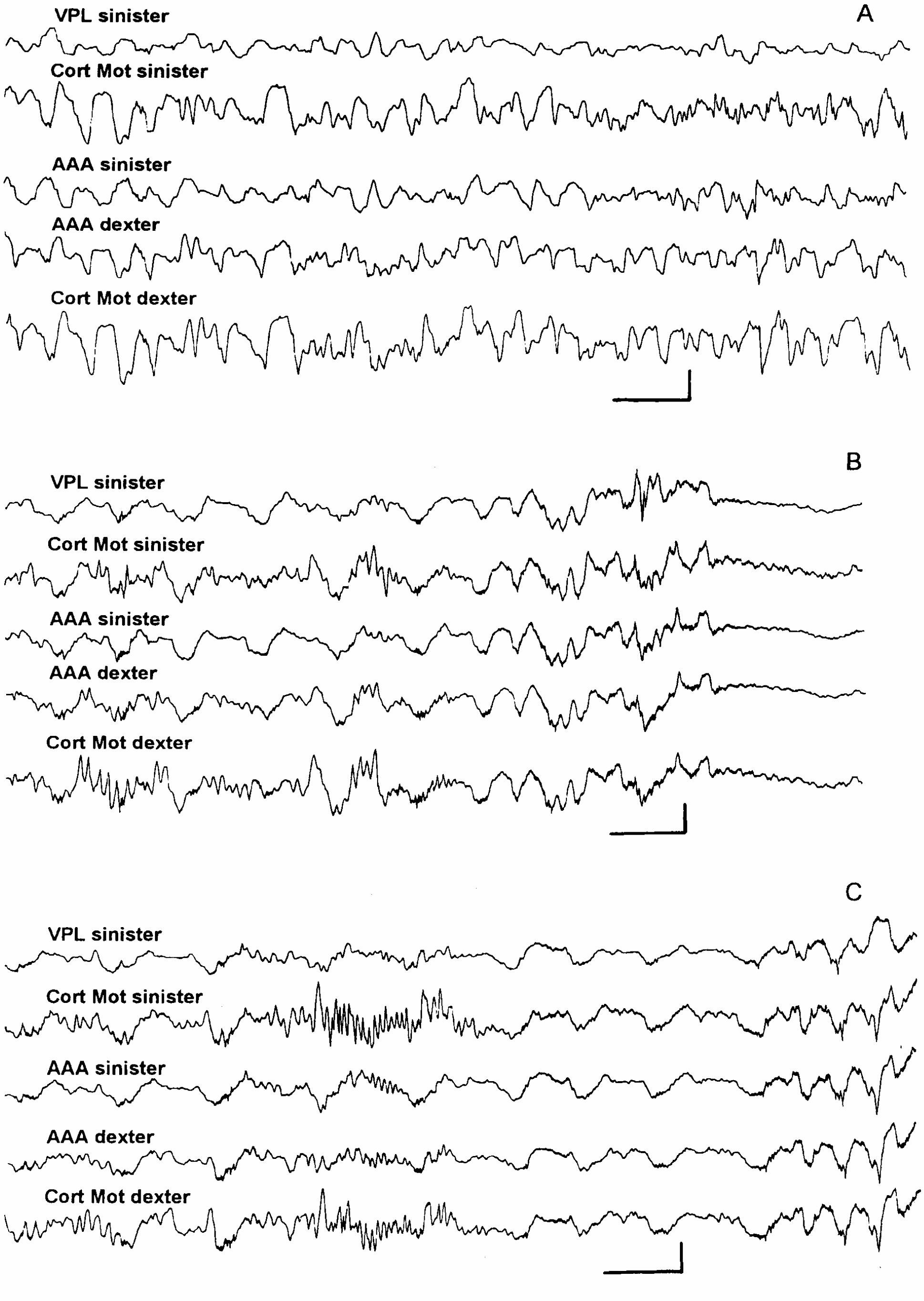


Fig. 1. Changes of rabbit brain bioelectrical activity under almost local exposure to a permanent magnetic field (PMF) south pole on AAA sinister. **A**, background activity; **B**, brain bioelectrical activity 6 to 7 minutes after beginning exposure to a PMF; **C**, brain bioelectrical activity 10 minutes after beginning exposure to a PMF. At the end of demonstrated curves, generalised hypersynchronous discharges are observed as potential complex—sharp waves and slow waves at 3Hz frequency. Calibration: horizontal bar represents 1 s, vertical bar 100 mkV. AAA, area amygdalarum anterior; VPL, nucleus ventralis posterior lateralis thalami; Cort Mot, motor cortex.

ity changes which had arisen due to local impact with the south pole of PMF had disappeared.

In an other series of experiments, when the north pole of the magnet-electrode was located in AAA, the brain bioelectrical activity was characterised by the appearance of slow activity, relatively high amplitude (up to 120 μV), and slow waves with frequency 2–3 Hz. These waves appeared in a random manner (see Figure 2B), and hypersynchronous slow activity of the brain was not recorded. Diminution of fast frequency band was observed.

The recording of brain bioelectrical activity was computerised, shown in Figure 3. Figure 3A represents the distribution curves of background frequency–amplitude of cortical and subcortical potential oscillation. It is obvious that almost all of the frequency–amplitude curves showed a minimal difference between the voltage values.

Figure 3B represents distribution curves of frequency–amplitude of cortical and subcortical potentials oscillations when AAA was affected locally by the south pole of the PMF. This exposure caused an increase of all frequency oscillation amplitudes. Moreover, a fast frequency band widened till 16 Hz. All of the frequency–amplitude curves demonstrate that changes of brain bioelectrical activity are generalised, because curves on figure 3B were calculated from background generalised hypersynchronous brain activity.

The pattern differed when the AAA was locally exposed to the PMF by a magnet-electrode north pole (see Figure 3C): general augmentation of amplitudes of slow frequency waves and general diminution of the high frequency band.

Data in Figure 4 show that changes in bioelectric potentials (in per cent) of every frequency band formed from impact

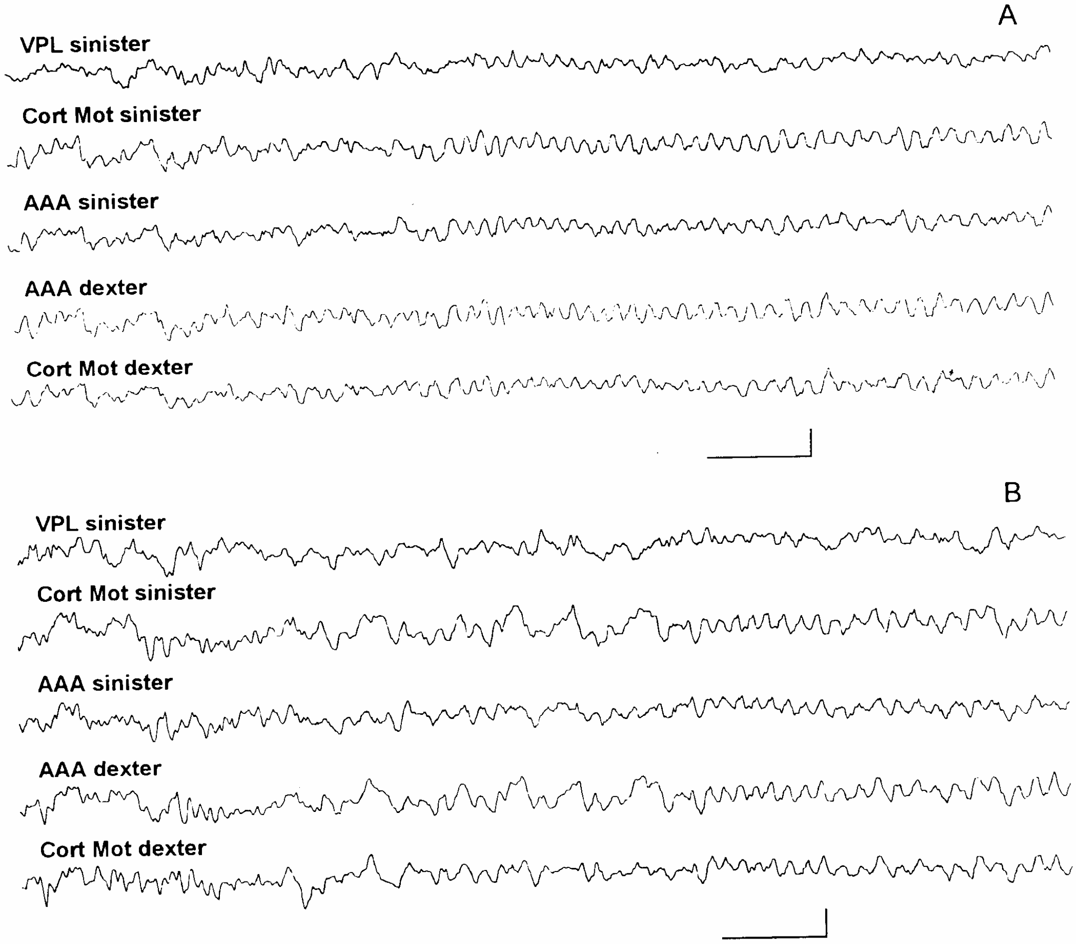
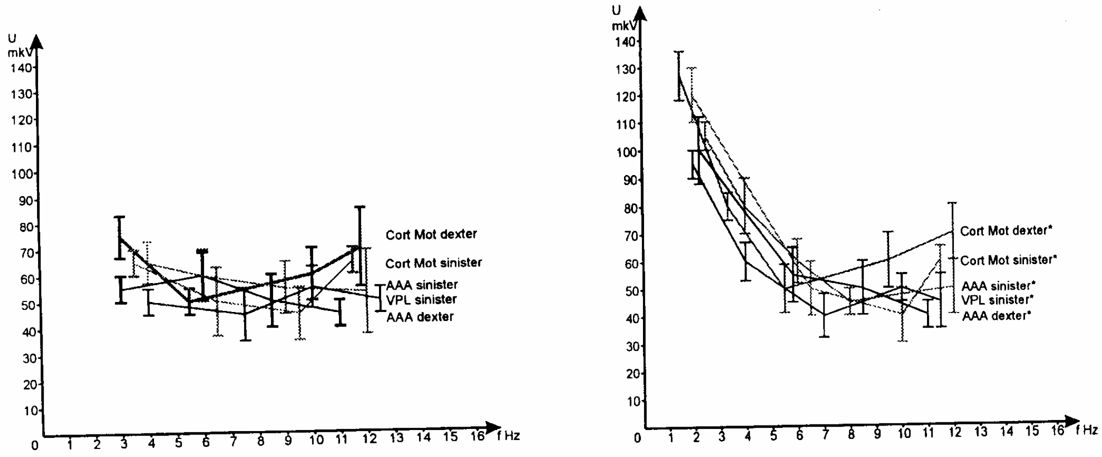


Fig. 2. Changes of rabbit brain bioelectrical activity under almost local exposure to a permanent magnetic field (PMF) north pole on AAA sinister. **A,** background activity; **B,** brain bioelectrical activity 6 to 7 minutes after beginning exposure to a PMF north pole. Calibration: horizontal bar represents 1 s, vertical bar 100 mkV. AAA, area amygdalarum anterior; VPL, nucleus ventralis posterior lateralis thalami; Cort Mot, motor cortex.



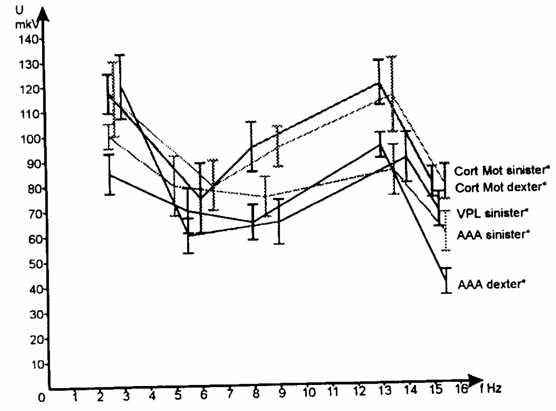


Fig. 3. Amplitude (U)–frequency (f) distribution curves before and during almost local exposure to a permanent magnetic field (PMF). Points on these curves represent mean values of frequency amplitudes (mkV), and vertical lines show average standard deviations. Activity changes due to a PMF are significant ($P<0.05$). **A**, background amplitude–frequency distribution curves of brain bioelectrical activity; **B**, amplitude–frequency distribution curves of brain bioelectrical activity caused by local exposure of AAA to the south pole of a magnet-electrode; **C**, amplitude–frequency distribution curves of brain bioelectrical activity caused by local exposure to a north pole of a magnet-electrode on AAA. * The changes were statistically significant ($P<0.05$) compared with background activity. AAA, area amygdalarum anterior; VPL, nucleus ventralis posterior lateralis thalami; Cort Mot, motor cortex.

with the PMF south pole are diametrically different to changes in bioelectrical activity of the north pole of the magnet electrode in AAA. Therefore, the functional state depends on the north or the south pole of the magnet-electrode placed in AAA.

During a single experiment on an animal, it was possible to alter the brain bioelectrical activity by changing the magnetic electrode pole in AAA (south to north or vice versa). The location of magnet-electrode tip on the left or right AAA was not the decisive factor for the determination of the brain functional state.

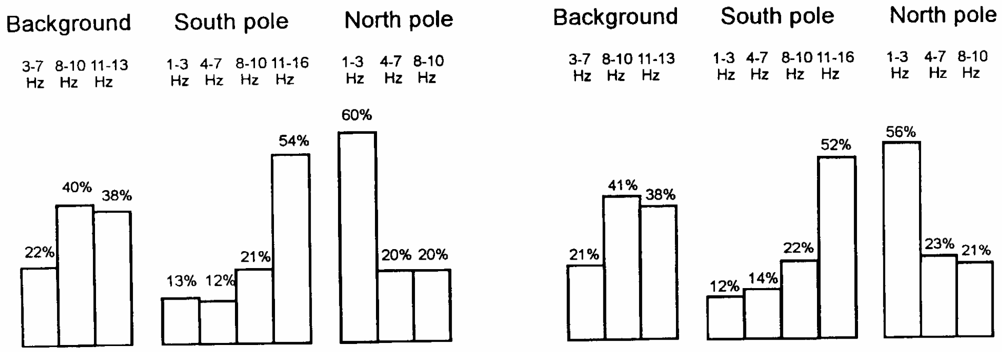
DISCUSSION

Brain bioelectrical activity parameters under a PMF exposure depended on the magnetic field pole on the electrode tip located in AAA, showing that the organisation of links in neuronal nets is influenced by the PMF, and that the nature of changes particularly depends on the magnetic induction vector direction. The nature of changes in rabbit brain bioelectrical activity under PMF exposure were due to a direct brain reaction to the field, and were not caused by stress or brain tissue damage, as the background activity in all experiments did not indicate irritation, spontaneous arousal reactions and outbursts of hypersynchronous potentials.

There are several explanations of the variable brain response to the PMF. It is known that a conductor, if it is placed in a magnetic field, tends to change its position according to magnetic induction vector direction and direction of current flow in the conductor. In living tissues, ion current flow functions as a conductor. Taking this into consideration, it is possible to raise the following hypotheses.

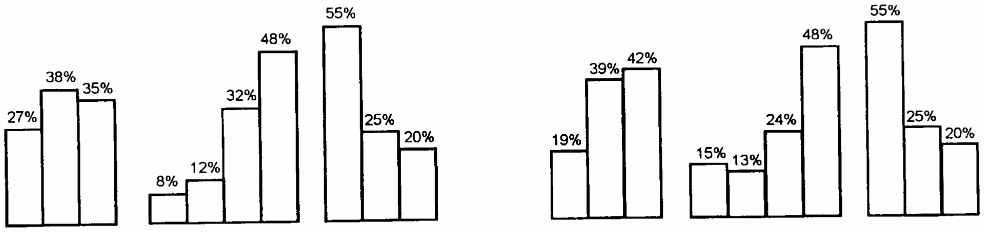
1. The mechanism of PMF action on brain activity may be explained by modulation of electronic gap junctional conductance. In such a case, conductance in the electrotonic gap will depend on the PMF induction vector orientation. There is some experimental evidence that such an electrotonic coupling is found in the frontal region of brain stem structures (Perez-Velazquez *et al.*, 1994).

2. The mechanism of the PMF effect on brain may be due to interactions with biomineralised iron corpuscles in brain frontal structures (Kirschvink *et al.*, 1985; Dobson and Grassi, 1996). The presence of ferromagnetic material in brain tissue also provides possible theoretical mechanisms



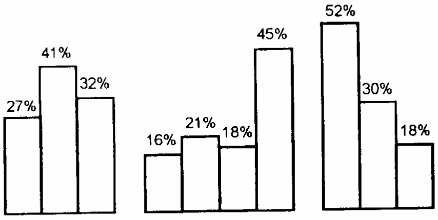
AAA sinister

AAA dexter



Cort Mot sinister

Cort Mot dexter



VPL sinister

Fig. 4. Proportions (%) of AAA waves of different frequency at cortical and subcortical leads during local exposure to permanent magnetic field. AAA, area amygdalarum anterior; VPL, nucleus ventralis posterior lateralis thalami; Cort Mot, motor cortex.

for the interaction of the PMF with the central nervous system depending on magnetic induction vector direction.

3. A more believable mechanism of PMF interaction with the central nervous system relates to the brain direct potential and the sensitivity of ion current flow to magnetic fields. The changes in EEG spectral and amplitude distribution caused by the PMF suggest that the fluxes of electric charge are turned away from their former isotropic distribution due to Lorentz forces exerted by the static magnetic field (Klitzing, 1991). These changes in brain bioelectrical activity are reflected by a deviation of ion current flow direction and deviation of brain direct current potential magnitude from its initial parameters. The above changes in turn have been observed to determine the distribution of excitatory and inhibitory states in brain neuronal nets (Русинов и Густсон, 1968; Русинов, 1969).

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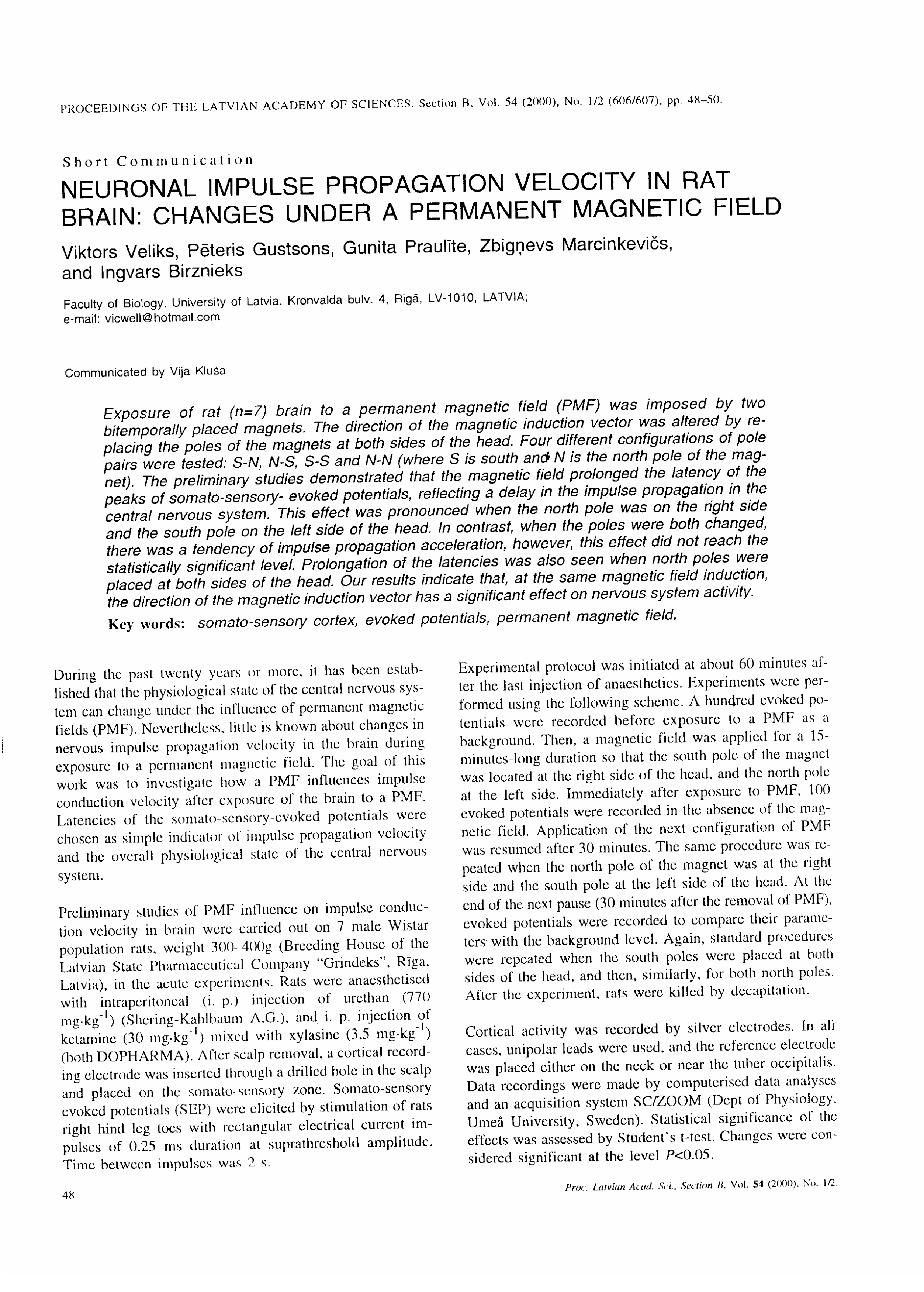
TRUŠA SMADZEŅU BIOELEKTRISKĀS AKTIVITĀTES PĀRMAIŅAS, IEDARBOJOTIES LOKĀLI AR PASTĀVĪGU MAGNĒTISKO LAUKU UZ AMIGDALĀRAJIEM KODOLIEM

Darba mērķis bija iegūt datus par neironu tīkla asociatīvo saišu aktivitātes regulācijas mehānismiem. Pētīta pastāvīgā magnētiskā lauka (PML) iedarbība uz galvas smadzenēm. Mūsu iepriekšējie pētījumi parādīja, ka, ievietojot galvas smadzenes PML, centrālās nervu sistēmas funkcionālā stāvokļa pārmaiņas ir atkarīgas no to orientācijas PML, t.i., no magnētiskās indukcijas vektora virziena attiecībā pret noteiktu smadzeņu daļu. Smadzeņu bioelektriskās aktivitātes pārmaiņas pētījām, iedarbojoties lokāli uz amigdalāro kodolu kompleksu (AKK) ar PML atsevišķi ar ziemeļpolu vai dienvidpolu. To panācām, ievadot AKK magnētu elektrodu, kura galos 1,5–2,0 mm rādiusā PML indukcija bija iedarbības sliekšņa līmenī. Gūtie dati hroniskos eksperimentos ar trušiem parādīja, ka smadzeņu bioelektriskās aktivitātes pārmaiņas PML iedarbības rezultātā ir atkarīgas no tā, kāds PML pols (ziemeļu vai dienvidu) atrodas amigdalā. Ziemeļpola iedarbībā notiek visas smadzeņu bioelektriskās aktivitātes svārstību palēnināšanās un lēna lielas amplitūdas asinhronu viļņu parādīšanās. Dienvidpola iedarbība rada pretēja rakstura pārmaiņas: parādās hipersinhrona, ģeneralizēta aktivitāte ar desinhronizācijas periodiem. Bioelektriskā aktivitāte atgādina epileptisko aktivitāti cilvēka elektroencefalogrammā.

9.4. Nervu impulsu vadīšanas ātruma izmaiņas žurku smadzenēs pastāvīga magnētiska lauka ietekmē



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Short Communication

NEURONAL IMPULSE PROPAGATION VELOCITY IN RAT BRAIN: CHANGES UNDER A PERMANENT MAGNETIC FIELD

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Exposure of rat (n=7) brain to a permanent magnetic field (PMF) was imposed by two bitemporally placed magnets. The direction of the magnetic induction vector was altered by replacing the poles of the magnets at both sides of the head. Four different configurations of pole pairs were tested: S-N, N-S, S-S and N-N (where S is south and N is the north pole of the magnet). The preliminary studies demonstrated that the magnetic field prolonged the latency of the peaks of somato-sensory- evoked potentials, reflecting a delay in the impulse propagation in the central nervous system. This effect was pronounced when the north pole was on the right side and the south pole on the left side of the head. In contrast, when the poles were both changed, there was a tendency of impulse propagation acceleration, however, this effect did not reach the statistically significant level. Prolongation of the latencies was also seen when north poles were placed at both sides of the head. Our results indicate that, at the same magnetic field induction, the direction of the magnetic induction vector has a significant effect on nervous system activity.

Key words: *somato-sensory cortex, evoked potentials, permanent magnetic field.*

During the past twenty years or more, it has been established that the physiological state of the central nervous system can change under the influence of permanent magnetic fields (PMF). Nevertheless, little is known about changes in nervous impulse propagation velocity in the brain during exposure to a permanent magnetic field. The goal of this work was to investigate how a PMF influences impulse conduction velocity after exposure of the brain to a PMF. Latencies of the somato-sensory-evoked potentials were chosen as simple indicator of impulse propagation velocity and the overall physiological state of the central nervous system.

Preliminary studies of PMF influence on impulse conduction velocity in brain were carried out on 7 male Wistar population rats, weight 300–400g (Breeding House of the Latvian State Pharmaceutical Company "Grindeks", Rīga, Latvia), in the acute experiments. Rats were anaesthetised with intraperitoneal (i. p.) injection of urethan (770 $mg \cdot kg^{-1}$) (Shering-Kahlbaum A.G.), and i. p. injection of ketamine (30 $mg \cdot kg^{-1}$) mixed with xylasine (3.5 $mg \cdot kg^{-1}$) (both DOPHARMA). After scalp removal, a cortical recording electrode was inserted through a drilled hole in the scalp and placed on the somato-sensory zone. Somato-sensory evoked potentials (SEP) were elicited by stimulation of rats right hind leg toes with rectangular electrical current impulses of 0.25 ms duration at suprathreshold amplitude. Time between impulses was 2 s.

Experimental protocol was initiated at about 60 minutes after the last injection of anaesthetics. Experiments were performed using the following scheme. A hundred evoked potentials were recorded before exposure to a PMF as a background. Then, a magnetic field was applied for a 15-minutes-long duration so that the south pole of the magnet was located at the right side of the head, and the north pole at the left side. Immediately after exposure to PMF, 100 evoked potentials were recorded in the absence of the magnetic field. Application of the next configuration of PMF was resumed after 30 minutes. The same procedure was repeated when the north pole of the magnet was at the right side and the south pole at the left side of the head. At the end of the next pause (30 minutes after the removal of PMF), evoked potentials were recorded to compare their parameters with the background level. Again, standard procedures were repeated when the south poles were placed at both sides of the head, and then, similarly, for both north poles. After the experiment, rats were killed by decapitation.

Cortical activity was recorded by silver electrodes. In all cases, unipolar leads were used, and the reference electrode was placed either on the neck or near the tuber occipitalis. Data recordings were made by computerised data analyses and an acquisition system SC/ZOOM (Dept of Physiology, Umeå University, Sweden). Statistical significance of the effects was assessed by Student's t-test. Changes were considered significant at the level $P<0.05$.

The PMF was obtained by samarium-cobalt fused magnets 20×20×10 mm in size. The magnetic field obtained by a samarium-cobalt magnet is permanent as this magnetic material does not change its physical parameters under the influence of temperature deviation and mechanical forces. This PMF, in contrast to an electro-magnetic constant field, does not fluctuate and lacks an electrical component. Figure 1 demonstrates the PMF parameters within a ¼ sphere section, as the magnetic field is symmetrical. Arrows represent the direction of the magnetic induction vector, and numbers correspond to the magnitude of induction (B). The application of PMF on rat brain was performed by two bitemporaly placed magnets. Four different configurations of the PMF were achieved by switching the magnetic poles at each side of the head. On the surface of brain cortex, the magnetic field induction was 100–110 mT, and it declined towards the midsagittal plane. This magnitude of PMF induction was used, since whole brain tissues have been shown to respond almost maximally to this field intensity (Easterly, 1982).

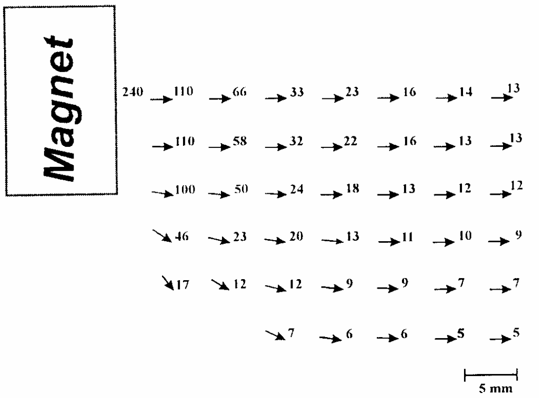

Fig.1. Permanent magnetic field (PMF) parameters of one magnet: direction of magnetic induction vectors is indicated by arrows (→), and numbers correspond to the magnitude of magnetic induction (B, mT). At the surface of the magnet, the magnitude of magnetic induction was 240–250 mT. The first line of arrows shows the magnitude of magnetic induction (100–110 mT) at the surface of rat brain.

After exposure to the PMF, the latency of primary response was changed, depending on the orientation of magnetic poles on both sides of the head. This effect could be observed usually only a few minutes after exposure to the PMF. To verify this, and to check whether anaesthesia influences the latencies in a time-dependent manner, we compared the background parameters of SEP with those during an experimental session, recorded 30 minutes after the two first configurations of magnetic field were tested: there was no difference between the latent period of SEP recorded before any application of PMF (14.0±0.9 ms) and SEP latencies, recorded 30 minutes after removal of the PMF (14.0+1.8 ms; $P>0.5$; Figure 2).

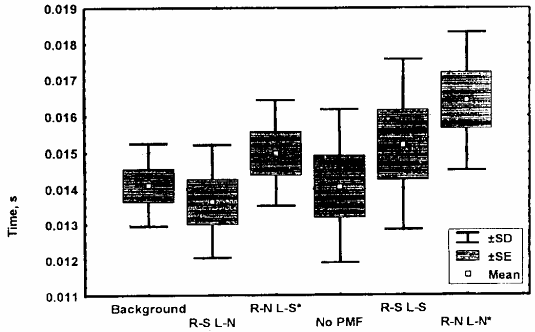

Fig.2. Somato-sensory evoked potentials (SEP) latency changes after permanent magnetic field (PMF) impact, using various combinations of magnet poles (N and S) and side of head (R and L). * statistically significant deviation from background levels.

Figure 2 shows that, when the south pole of the magnet was at the right side of the head, and the north pole at the left side, the cortical SEP latencies tended to decrease, but this effect did not reach the statistically significant level. When poles were exchanged (the north pole of the magnet at the right side and the south pole at the left side of the head), a statistically significant prolongation of the SEP latencies was observed ($P<0.05$). When similar poles of magnets were placed at each side of the head, a prolongation of the SEP latency was observed, but a significant effect was observed only for the north poles ($P<0.05$).

A possible mechanism by which a PMF exerts an effect on the nervous system may be via the cell membrane lipid, since higher phospholipid and protein macromolecular compound content increases sensitivity to a PMF. The movement of charged particles in the blood circulatory system may also be significantly altered by magnetic fields (Liburdy and Tenforde, 1986; Colbec *et al.*, 1986; Ueno and Iwasaka, 1996). Another mechanism of PMF action on brain may be by changing the intensity of reactions for synthesis and release of some neurotransmitters (Young, 1969). It has been shown that a PMF with induction 0.27 T increases acetycholinesterase activation by up to 40 %, and at 1.7 T by 250 % (Easterly, 1982).

The experimental results demonstrated that a magnetic field can prolong the latency of the peaks of somato-sensory-evoked potentials, indicating a delay in impulse propagation in the central nervous system. This effect was pronounced when the north pole was at the right side and the south pole at the left side of the head. In contrast, with the opposite orientation of the poles, we observed a tendency of acceleration of impulse propagation, but this effect did not reach a statistically significant level. Prolongation of the latencies was also observed when the north poles were placed at both sides of the head. Our results indicate that, at a similar induction of magnetic field, the direction of the magnetic induction vector has a significant effect on the activity in the nervous system. The experiments showed that our experimental design and stimulation parameters were suitable for demonstration of the influence of a PMF on the central nervous system. The data indicate that research in this direction should be continued. Further specific experiments will be designed and elaborated.

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NERVU IMPULSU VADĪŠANAS ĀTRUMA IZMAIŅAS ŽURKU SMADZENĒS PASTĀVĪGA MAGNĒTISKĀ LAUKA IETEKMĒ

Ir zināms, ka centrālās nervu sistēmas funkcionālo stāvokli var ietekmēt ar pastāvīgu magnētisko lauku (PML). Mūsu mērķis bija noskaidrot, kā mainās signāla izplatīšanās ātrums centrālajā nervu sistēmā pēc smadzeņu īslaicīgas atrašanās PML, atkarībā no to orientācijas. Akūti eksperimenti tika veikti ar žurkām. Kā kritērijs PML iedarbības efekta konstatēšanai tika izvēlēti izsaukto somatosensoro potenciālu latentie periodi. Mūsu rezultāti norāda, ka vienādas indukcijas PML nervu sistēmu var ietekmēt dažādi atkarībā no lauka magnētiskās indukcijas vektora virziena.

9.5. Pastāvīga magnētiska lauka ietekme uz žurkas smadzeņu funkciju pēc sirds darbības izmaiņām

Bioelectromagnetics 25:211–215 (2004)

Static Magnetic Field Influence on Rat Brain Function Detected by Heart Rate Monitoring

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The aim of the present study was to identify the effects of a static magnetic field (SMF) on rat brain structures that control autonomic functions, specifically heart rate and heart rhythmicity. The experiments were carried out on 44 male Wistar rats under ketamine–xylazine anesthesia. SMF was induced using samarium-cobalt fused magnets (20 × 20 × 10 mm in size) placed bitemporally. Magnetic induction intensity was 100 mT on the surface of the head. Duration of magnetic field application was 15 min. An electrocardiogram was recorded from limb lead II, and both heart rate (average duration of cardiac cycles) and heart rhythmicity were analyzed before and after SMF application. SMF evoked changes in both heart rate and rhythm in 80% of the animals; the predominant effects were bradycardia and disappearance of respiratory sinus arrhythmia. However, the effectiveness of SMF in large measure depends on both functional peculiarities and functional activities of brain autonomic centers. Bioelectromagnetics 25:211–215, 2004. © 2004 Wiley-Liss, Inc.

Key words: heart rhythmicity; autonomic nervous system; ECG; brain

INTRODUCTION

Several brain activities may be influenced by weak electromagnetic fields [Repacholi and Greenbaum, 1999]. Static magnetic fields (SMF), alone or in combination with extremely low frequency magnetic fields (ELF MF) having an intensity of approximately 1 mT, influence the kinetics of appropriate cell signaling pathways [Tofani et al., 2001]. Specifically, weak magnetic fields may have an influence on calcium homeostasis in different cells, including neurons and astrocytes, via voltage gated ion channels of the cell membrane [Fanelli et al., 1999; Pessina et al., 2001], on Na–K-ATPase and cytochrome oxidase activities, and on clustering of membrane proteins and their interaction [Golfert et al., 2001].

Clinical observations reveal variable effects of magnetic fields on human autonomic nervous system and heart frequency control mechanisms [Sastre et al., 1998; Graham et al., 2000]. These clinical observations suggest that the functional state of the central nervous system could change under the influence of SMFs. Furthermore, the selective effects of the two opposite directions of SMF applied to the brain are well known [Gustson et al., 1982]. We therefore attempted to detect changes in both heart rate and heart rhythm as indicators of SMF induced functional changes in brain structures that maintain autonomic control of excitation and conduction in the heart.

MATERIALS AND METHODS

The experiments were carried out on 44 male Wistar rats (weight 300–400 g). Analgesia was induced by intraperitoneal (i.p.) injection of ketamine (30 mg/kg) with xylazine (3.5 mg/kg). Animals were obtained from Gailezers Hospital (Riga) vivarium 3 days prior to experimentation. They were housed under standard laboratory conditions with natural daylight cycles, and standard laboratory food and water were available ad libitum. Experiments were performed according to guidelines of the European Ethics Committee for Laboratory Animal Science, with experimental protocol approval by the Latvian LAS Ethics Committee.

Two electrocardiograms (ECG) were recorded from limb lead II. The first recording was taken immediately before SMF exposure, with the second

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TABLE 1. Statistically Average Values of the Duration of the Cardiac Cycle (R–R Interval) in the Control and Treatment Groups

Subject groups	Values of R–R intervals before SMF application (mean ± SD)	Values of R–R intervals after SMF application (mean ± SD)
Control	0.232 ± 0.025 s	0.231 ± 0.027 s
rN-lS	0.245 ± 0.028 s	0.247 ± 0.039 s
rS-lN	0.236 ± 0.019 s	0.248 ± 0.024 s*
rS-lS	0.242 ± 0.017 s	0.253 ± 0.025 s*
rN-lN	0.264 ± 0.057 s	0.263 ± 0.066 s

Abbreviations for group designations—rN-lS: the North pole of the magnet is on the right side of the head and the South pole is on the left side; rS-lN: the orientation of SMF in the opposite direction; rS-lS: the South poles are located bitemporally; rN-lN: the North poles are located bitemporally.
*Significantly different from paired control values ($P < .05$, *t*-test).

of the heart rate after magnetic field exposure was not statistically different from pre-exposure values (Table 1). The reason for this is that every group contained animals with variable sensitivity towards SMF (Table 2). However, looking at subjects individually, SMF application to the head evoked heart rate changes in 28 rats; the average heart rate in seven animals did not change.

The largest response observed in 24 rats was a deceleration of heart rate (bradycardia). The relative amplitude of evoked deceleration of heart rate was on the average a 7–9% decrease from the heart rate value before SMF application (Table 3). Conversely, four rats demonstrated an increase in heart rate (by an average of 12.7 ± 10.6%). The average heart rate before and after SMF exposure was compared for each animal individually (within-subjects design), evaluating the statistical significance of the differences ($P < .05$), which was the criterion used to ascertain whether the frequency decreases (bradycardia), increases (tachycardia), or remains unchanged after SMF exposure.

TABLE 2. Experimental Groups and the Different Patterns of Response to SMF

Treatment groups	Total no. of animals in each group	No. of animals with characteristic changes in heart rate evoked by SMF exposure		
		Bradycardia	Tachycardia	No changes
Control	9	5	4	0
rN-lS	10	6	1	3
rS-lN	8	6	0	2
rS-lS	9	8	1	0
rN-lN	8	4	2	2

Abbreviations for group designations—as in Table 1.

TABLE 3. Relative Amplitudes of Bradycardia Evoked by SMF

Treatment groups	Relative amplitudes (mean ± SD) of heart rate deceleration (%)
Control (without SMF)	2.9 ± 2.4
rN-lS	9.5 ± 2.9*
rS-lN	7.0 ± 2.8*
rS-lS	6.2 ± 5.0
rN-lN	7.5 ± 5.1

Abbreviations for group designations—as in Table 1.
*Significantly different from paired control values ($P < .05$, *t*-test).

The spectral analysis of R–R intervals (one 5 min long ECG recording includes more than 1000 consecutive R–R intervals) revealed certain dominant frequencies (Fig. 2) in 25 spectrograms recorded before SMF application. In all spectrograms, the largest maximum of spectral density in physiologically significant range corresponded to the wavelength representing 4–6 cardiac cycles. That peak of spectral density disappeared under the influence of SMF (Fig. 3).

A strongly pronounced peak of spectral density was absent before SMF applications in the cardiospectrograms of 10 experimental rats. All four animals whose reaction to SMF application was an acceleration (tachycardia; Table 2) of heart rate were members of this group.

DISCUSSION

Both autonomic nervous system branches, the parasympathetic and sympathetic nerves, modulate cardiovascular function and are tonically active in regulating heart function.

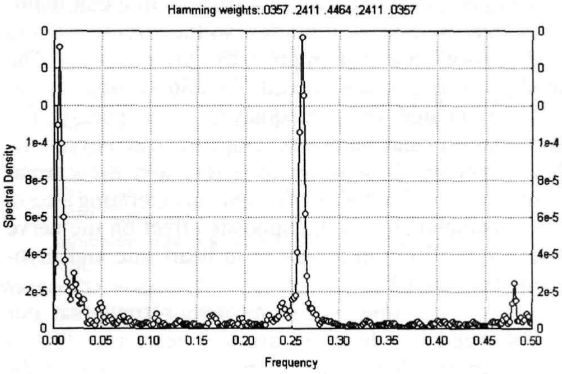

Fig. 2. Example of cardiospectrograms of anesthetized rats (control group). Frequency is the inverse of wavelength, which is expressed as a number of cardiac cycles per wave.

recording taken immediately after exposure. Recording duration was 5 min in each case. Durations of all R–R intervals in both recordings were measured, and data were used for spectral analysis and calculation of mean duration of cardiac cycle (mean heart rate).

A magnetic field was applied by means of two samarium-cobalt fused magnets 20 × 20 × 10 mm in size located bitemporally (on both sides of the head). The SMF was strictly symmetrical with the average value of the magnetic induction intensity approximately 250 mT at the surface of the magnet (measurements were made at 16 separate points on the magnets' surfaces), 100 mT on the surface of the cortex and 50 mT in the core structures of the brain. The direction of the magnetic induction vector was changed by varying magnet poles on both sides of the head. We used teslaampermeter Φ4354/1, USSR standard ГОСТ 5.1977-73) for SMF strength measuring. Figure 1 illustrates the intensity of SMF (mT) in five planes located between the poles of two magnets. Distance between the magnets' surfaces is 4 cm; distance between measured planes is 1 cm.

Rats were divided in five groups. The first group ($n = 9$) was the control condition, where rats were not exposed to the SMF. Rats in the four treatment (experimental) groups were exposed to the SMF. These treatment groups were classified according to the direction of the SMF vector:

- Treatment group rN-lS ($n = 10$): where the magnet's North pole was located on the right side of rat's head and South pole was on the left side;
- Treatment group rS-lN ($n = 8$): where the magnet's South pole was located on the right side of rat's head and North pole was on the left side;
- Treatment group rS-lS ($n = 9$): where the magnet's South poles were located bitemporally;
- Treatment group rN-lN ($n = 8$): where the magnet's North poles were located bitemporally.

The duration of exposure in each treatment group was 15 min. The control animals simply rested for 15 min between ECG recordings.

Data were recorded by means of a computerized data acquisition system SC/ZOOM (Department of Physiology, Umea University, Sweden). The effects of the magnetic field on heart rate and rhythm were studied by spectral analysis.

Spectral analysis was done of wave-like fluctuations of R–R intervals. The number of cardiac cycles per one wave was used as a unit of measurement for wavelength. The frequency (inverse of the wavelength of observed fluctuations of heart rate) was calculated. Spectral analysis was accomplished using Fourier transforms (Statistica v5.5 StatSoft, Inc.). Differences in mean heart rate between control and SMF exposed animals were determined by paired Student's *t*-test (two-tailed); differences were considered significant at $P < .05$.

RESULTS

In some of the treatment groups, depending on the direction of the applied SMF vector, the average value

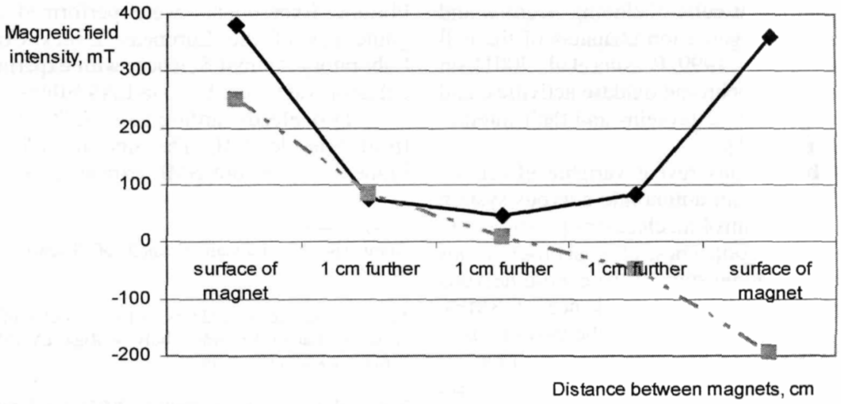

Fig. 1. Spatial distribution of magnetic field intensity between magnets along the lines connecting the central points of the magnets' surface. —■—■—: Opposite polarity magnets (N S; S N); – – ■– –■– –: same polarity magnets (N N; S S);—designation + and − indicate direction of magnetic vector.

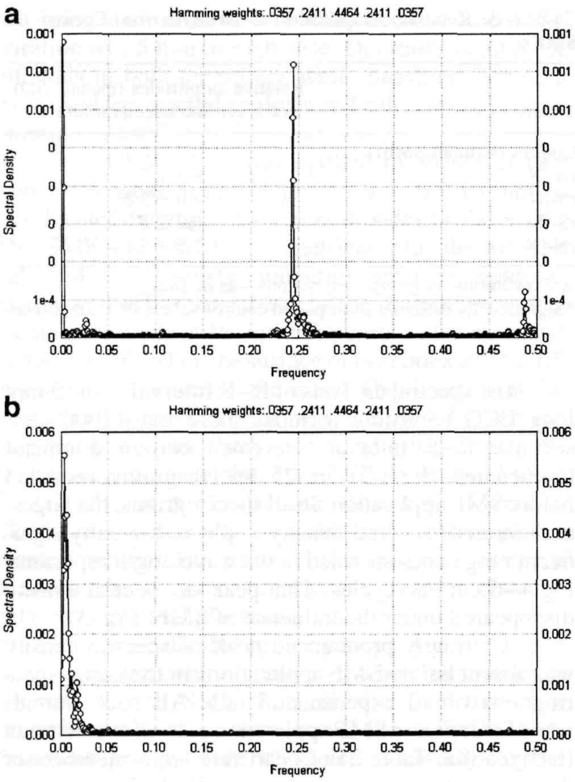

Fig. 3. Cardiospectrograms of the anesthetized rat before (**a**) and after (**b**) the exposure of the animal's head to static magnetic field (SMF). Frequency is the inverse of wavelength, which is expressed as a number of cardiac cycles per wave.

The tonic activity of the autonomic nervous system in the cardiovascular system is due to the combined effect of activity in three interrelated areas of the medulla and hypothalamus. The medulla contains a vasomotor area comprised of a pressor area that maintains tonic sympathetic outflow to the circulation and a depressor area that inhibits the pressor area. The medulla also contains a cardioinhibitory area which, when stimulated, simultaneously increases parasympathetic activity and decreases sympathetic activity to the heart, thereby decreasing heart rate and myocardial contractility. Stimulation of a cardioaccelerating area in the hypothalamus has the opposite effect on the nerve outflow, resulting in increases in heart rate and myocardial contractility.

Our experimental results demonstrate that our anesthetized rats have an average heart rate that is characteristic for small homeothermic mammals, approximately 250 beats/min. There is no evidence that the anesthesia used in the experiments by itself caused consequent changes in the animals' cardiac function. This is confirmed by the control group ECG data, recorded with a 15 min interval (see Table 1): the statistically average values, standard deviations, and coefficients of variability of the cardiac cycle in both periods were practically identical.

The long term sequences of cardiac cycles in 71% of all rats used in our experiments revealed periodic fluctuation in cycle duration. It is known that changes in heart rate are affected through a reciprocal action of both the branches of the autonomic nervous system. Vagal release of acetylcholine in the SA and AV nodes decrease SA firing rate and hence heart rate, while decreasing conduction velocity through the AV node. Sympathetic stimulation has the opposite effect. Small variations in cycle length are seen normally in resting individuals. This variability has been suggested to reflect physiological oscillations in sympathetic and parasympathetic tone [Wagner and Persson, 1998].

The dominant duration of each wave of fluctuations in cardiac cycle length in anesthetized rats in our experiments was approximately 1.0–1.5 s (4–6 cardiac cycles), which corresponded to the duration of one respiratory cycle. It is known that changes in the sinus rate that accompany normal respiration, called *respiratory sinus arrhythmia* (RSA), alter cycle length without affecting impulse propagation through the atria, AV node, and ventricles [Kastor, 1994]. RSA is most likely caused when stimuli originating in stretch receptors in the lungs and on the chest wall alter the delicate balance between the sympathetic and parasympathetic influences on the SA node. Heart rate accelerates during inspiration because of decreased vagal tone and increased sympathetic tone, whereas increased vagal tone and decreased sympathetic tone slow the sinus pacemaker during expiration.

Therefore, the presence of respiratory sinus arrhythmia in the majority of our experimental animals suggests a predominance of parasympathetic tone in hypothalamic and medullary cardiovascular centers relative to the activity in sympathetic central neurons of anesthetized rats. The absence of RSA, i.e., relatively low activity of vagal output, was revealed in 10 animals prior to SMF application and in all experimental rats after SMF influence.

SMF application evoked changes in heart rate (bradycardia or tachycardia) in approximately 80% of rats; the others animals demonstrated weak (insignificant) responses or did not react at all. Other investigators also report conflicting findings. Sastre et al. [1998] demonstrated an alteration in heart rate variability when subjects slept under the influence of intermittent 60 Hz magnetic fields, but no effect of a continuous 16.7 Hz magnetic field on human heart rate [Griefahn et al., 2001]. Considering the present results and the available literature, it is very likely that both differential sensitivity and differential reactivity of individual animals to SMF is due to the ability of magnetic fields to influence large numbers of specific biological "targets"—cells (neurons, astrocytes, endocrine cells), biochemical reactions, and different mechanisms of intercellular communication [Arutiunian et al., 1998; Pessina et al., 2001; Tofani et al., 2001].

Most frequently, the reaction in our experiments evoked by SMF treatment included two obvious components: deceleration of heart rate and disappearance of RSA. We hypothesize that such discrepant effects are evidence of independent influences of SMF on two different structures of autonomic nervous system: (1) Inhibition of neurons in the cardioaccelerating area of the hypothalamus and (2) Inhibition of neurons in medulla which regulate the reciprocal influences of respiration to pacemaker cells in the heart. Presumably, the effect of SMF on the rat brain also depends on the level of tonic activity of both centers. This assumption was confirmed by the results in four animals having relatively low initial excitability of medullary parasympathetic centers, since RSA was absent, prior to electromagnetic exposure: the effect of SMF was acceleration of the heart rate, instead of the usual deceleration.

The results of our experiments suggest that the direction of the SMF vector relative to the anatomic projection of brain structures maintaining autonomic control of heart rate is not of great importance. It is not unlikely that other structures or neural mechanisms may be sensitive to field direction. For example, clinical observations suggest that the effect of SMF used in complex medical treatment of epilepsy depends on the direction of the magnetic field vector relative the patient's head [Gustson et al., 1982]. One interpretation of the current data is that many different factors—the SMF parameters (strength, direction, duration of exposure), functional peculiarities of regulatory mechanisms, state of health—may be important variables in determining whether SMF exposure will affect brain functions. The results of various studies by numerous investigators on the effects of SMF on the brain in general support the view that the physical mechanisms of these effects are complex and, possibly, accomplished on many different levels: molecular, membrane, cellular, and intercellular communication.

Our experimental results indicate that (1) the application of SMF to an anesthetized rat's head can influence the higher centers (hypothalamic?) of autonomic regulation of heart function and that (2) the nature of this influence can be dependent on the relative equilibrium characteristics of the sympathetic and parasympathetic centers. To confirm this view, it would be appropriate to systematically evaluate the effect of SMF on an animal's brain by measuring changes in various neurotransmitter systems. To this end we have begun such an investigation.

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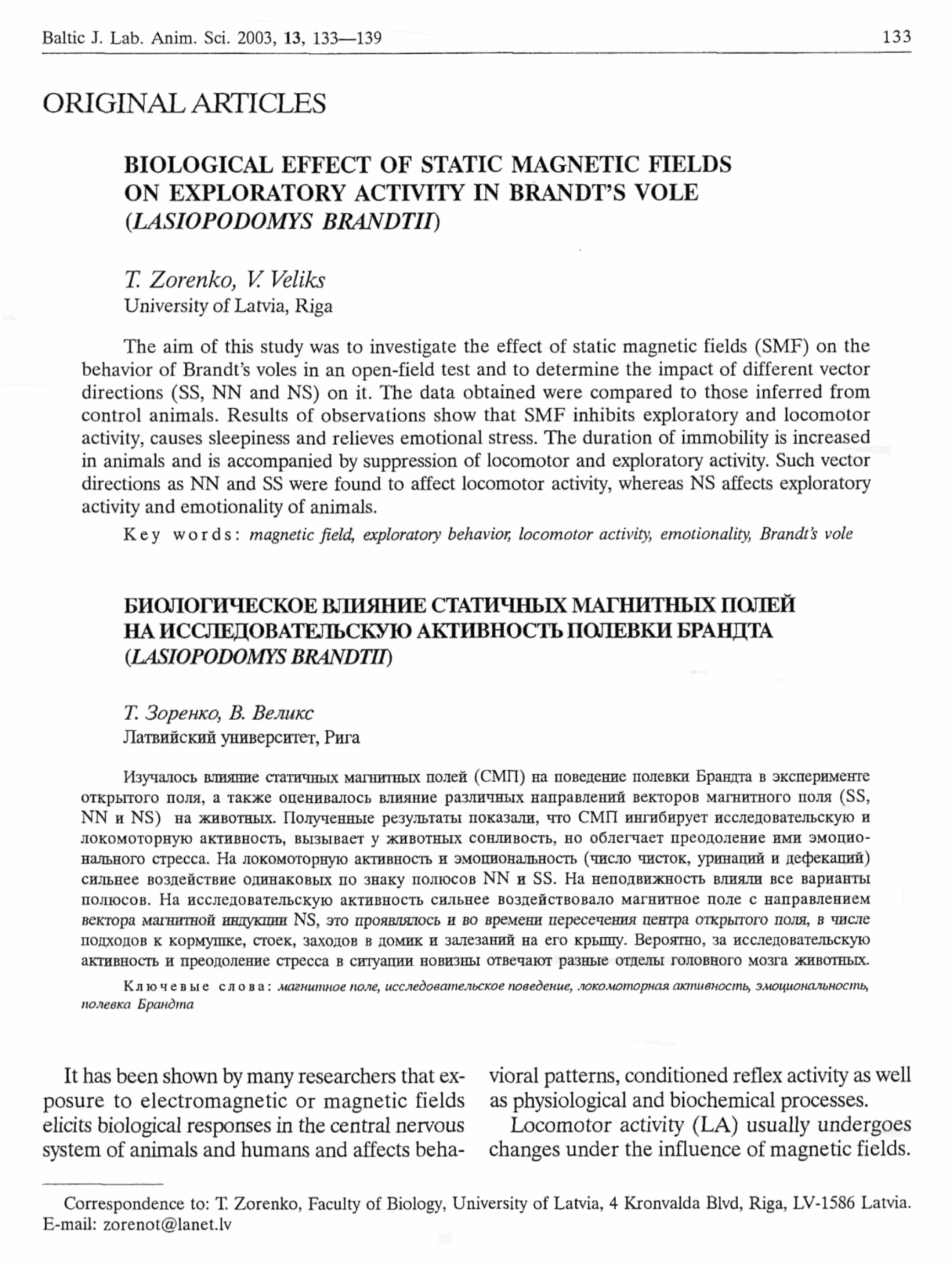
9.6. Pastavīga magnētiska lauka bioloģiska ietekme uz lauku peles (Lasiopodomys Brandtii) izpētes aktivitāti



Baltic J. Lab. Anim. Sci. 2003, **13**, 133—139 133

ORIGINAL ARTICLES

BIOLOGICAL EFFECT OF STATIC MAGNETIC FIELDS ON EXPLORATORY ACTIVITY IN BRANDT'S VOLE (*LASIOPODOMYS BRANDTII*)

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The aim of this study was to investigate the effect of static magnetic fields (SMF) on the behavior of Brandt's voles in an open-field test and to determine the impact of different vector directions (SS, NN and NS) on it. The data obtained were compared to those inferred from control animals. Results of observations show that SMF inhibits exploratory and locomotor activity, causes sleepiness and relieves emotional stress. The duration of immobility is increased in animals and is accompanied by suppression of locomotor and exploratory activity. Such vector directions as NN and SS were found to affect locomotor activity, whereas NS affects exploratory activity and emotionality of animals.

Key words: *magnetic field, exploratory behavior, locomotor activity, emotionality, Brandt's vole*

БИОЛОГИЧЕСКОЕ ВЛИЯНИЕ СТАТИЧНЫХ МАГНИТНЫХ ПОЛЕЙ НА ИССЛЕДОВАТЕЛЬСКУЮ АКТИВНОСТЬ ПОЛЕВКИ БРАНДТА (*LASIOPODOMYS BRANDTII*)

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Изучалось влияние статичных магнитных полей (СМП) на поведение полевки Брандта в эксперименте открытого поля, а также оценивалось влияние различных направлений векторов магнитного поля (SS, NN и NS) на животных. Полученные результаты показали, что СМП ингибирует исследовательскую и локомоторную активность, вызывает у животных сонливость, но облегчает преодоление ими эмоционального стресса. На локомоторную активность и эмоциональность (число чисток, уринаций и дефекаций) сильнее воздействие одинаковых по знаку полюсов NN и SS. На неподвижность влияли все варианты полюсов. На исследовательскую активность сильнее воздействовало магнитное поле с направлением вектора магнитной индукции NS, это проявлялось и во времени пересечения центра открытого поля, в числе подходов к кормушке, стоек, заходов в домик и залезаний на его крышу. Вероятно, за исследовательскую активность и преодоление стресса в ситуации новизны отвечают разные отделы головного мозга животных.

Ключевые слова: *магнитное поле, исследовательское поведение, локомоторная активность, эмоциональность, полевка Брандта*

It has been shown by many researchers that exposure to electromagnetic or magnetic fields elicits biological responses in the central nervous system of animals and humans and affects behavioral patterns, conditioned reflex activity as well as physiological and biochemical processes.

Locomotor activity (LA) usually undergoes changes under the influence of magnetic fields.

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These changes depend on magnetic flux density: the LA of animals increases at 50 mT, whereas in the 100 to 400 mT range a reduction is noted [1, 9]. LA in fish was found to increase by 50–300% in 64% of cases, compared to the background activity, and these changes differ in individual specimens. Special experiments with birds show that in 68% of cases LA rises by more than 100%, compared to the background level, in response to 0.07 mT magnetic field [10].

The LA of mice measured in experiments using long-time exposures (up to 72 h) revealed no significant differences in the immediate LA between control and exposed mice [4]. Other experiments (exposure to 0.5 Hz magnetic field at 1–2 mT intensity) showed an increase in the activity of rats in an open-field test [11].

It has been clearly demonstrated that the locomotor activity and emotional reactions of rats undergo changes under the influence of magnetic fields during prenatal ontogenesis. These alterations are more manifest in male animals and involve not only behavioral changes but also changes in their total weight and that of the testes and hypophysis. Prolonged exposure to a low-intensity field also causes a reduction in LA. Animals show significant changes in associative learning and their ability to memorize (Riskanova, 1980: cit. [9]), the latent period of conditioned reflex development being increased.

As revealed by experimental studies conducted on animals, the observed effects and the character of biological responses largely depend on the orientation of static magnetic fields (SMF). Our previous studies on animals [6, 13] and clinical observations with epilepsy patients [7] suggest that vector direction is one of the main factors affecting the magnitude of responses evoked in the organism. The character of neuron reactions largely depends on magnetic field orientation. These reactions in the sea skate were found to occur in the acoustic lateral part of the brain, one group of neurons responding actively to the south pole and being inhibited during exposure to the north pole, while another group responding in an opposite manner [3]. This phenomenon has anatomical genesis and is due to the bilateral symmetry of *Lorencini ampullae*. This anatomical structure apparently is responsible for the mosaic of activaton and inhibition process in the CNS that allow to separte an effect of magnetic field from other irritation sources.

Although experimental animals have been widely used as a source of information in examining the effects of magnetic fields on orientation, homing and navigation reactions, the mechanism of these phenomena still remains obscure.

The purpose of the present study was to investigate the behavior of animals in an open-field test and to determine how different SMF directions affect their behavioral patterns.

MATERIAL AND METHODS

Brandt's vole (*Lasiopodomys brandtii*) (*Rodentia*) was chosen as a test animal because of its high exploratory activity and the ability to overcome easily stress caused by a new situation. The exploratory activity and behavior of Brandt's vole in an open-field test was investigated previously [14].

Progenitors of Brandt's vole colony at the University of Latvia were captured in Chita steppes (Russian Federation, Southern Siberia). This colony has five years breeding history in vivarium. Healthy animals were maintained in plastic cages at constant room temperature (20±2 °C) and 14L/10D light–dark cycle. Their basic nutrition was vegetables and a mixture of grain.

90 to 120 days old males in the 6th to 8th generation were selected for open-field experiments. Prior to test each animal was placed in a small-size 8×4×5 cm Plexiglass cage and exposed to SMF for 15 min at the surface magnetic flux density 250 mT generated by 20×20×10 mm samarium–cobalt fused magnets. The effects of SMF on animal brain were studied by placing two magnets on both sides of the cage. Three different configurations of SMF magnetic induction orientation were applied by switching the magnets' poles on each side of the cage at the level of the animal head: south poles (SS) or north poles (NN) on both sides of the cage, south pole on the left side of the cage and north pole on the right side of the cage (SN). We could only use three

SMF configurations because the animals have a comparatively high freedom of movement in small cages. A magnetic field was applied by means of two samarium–cobalt fused magnets 20×20×10 mm in size located bitemporally (on both sides of the head). The SMF was strictly symmetrical, with magnetic induction intensity on a surface of magnets 250mT, on the surface of the cortex 100 mT and in the core structures of the brain 50 mT. The direction of a magnetic induction vector was changed by varying magnet poles on both sides of the head. We used tesla-ampermeter Ф4354/1 (USSR standard ГОСТ 5.1977-73) for SMF strength measuring [13].

The treatment and control groups consisted of 22 and 16 male animals, respectively, each animal being subjected to an open-field test for 5 min. The open field consisted of a circle (1 m in diameter) with a food container (7 cm in diameter) with grain placed in the center and a 100 W filament lamp at the height of 70 cm above the surface. The open-field test area was divided into 20 cm by 20 cm squares. Between the tests the box and the apparatus were cleaned by using a detergent and 40% ethanol and allowed to dry. Each vole was taken from its home cage and placed onto the open field in a transparent 15×15×10 cm Plexiglass box with two exits. The latency (Lat) to leave the box was measured along with other behavioral parameters.

The following abbreviations will be used below: Loco = locomotor activity, i.e., the number of squares traversed during each minute (Min1, Min2, Min3, Min4, Min5) and in total 5 min; Firstcom = the time of the first coming to the food container in the center of the open field or center-crossing time; N com = the number of comings; Groom and Grumdur = the number and time of autogrooming (all preening, scratching, licking or biting) recorded for each animal; Immobile = the time of immobility for each animal in the box or outside it; Standsup = standing on hind paws with support on the walls and box; Standwsup = standings without support on the walls; Tostand = total number of standing; Dig = digging. Each stopping in the box (In box) and climbing upon it (On box), urination (Urin), defecation (Def), digging and sneezing (Sneeze) were counted too.

In addition, total exploratory activity (EA) of each experimental animal was evaluated, taking into consideration such patterns of open field exploration as walking, scratching at the floor or walls with the forepaws, stopping and sniffing in the box and climbing upon it and exploration of objects. Total exploratory activity was measured in points: the most active animals scored 2, mid active – 1 and the passive ones – 0 points.

Since it can be assumed that the animal's behavior in the second experiment (one after another) would be different from that in the first experiment as a result of acquiring individual experience and information on the structure of the "open field", three more experimental series were additionally devised to explore: a) the influence of SMF in the 1st experiment ($n=10$), b) the influence of SMF in the 2nd experiment ($n=10$), and c) two experiments were conducted without magnet ($n=5$). Repeated experiments were conducted at one-week intervals.

One-way analysis of variance (ANOVA) was used to assess the results.

RESULTS

SMF INFLUENCE ON BEHAVIOR

The results obtained show that SMF affects the behavior of animals in the open-field test as follows (Table). Control animals, unlike the experimental ones, usually leave the box immediately at the beginning of the test. The experimental vole becomes immobilized either in the box (and hence the latency increases), or near the wall (immobility duration increases up to 100–200 s). Locomotor activity is suppressed, too, and this suppression is observed during each minute of the test: the analysis of variance revealed highly significant differences between the two groups with respect to the total number of entered squares. During the first minute the locomotor activity decreased to 32.6%, during the second and third minute to 33.0% and 14.7% (the differences are not statistically significant), during the fourth and fifth minute to 45.4% and 26.2%, respectively. Total LA was below 31.3%.

Our experiments revealed that the locomotor activity of animals exposed to SMF was signifi-

Table/Таблица

Some Brandt's voles behavior parameters obtained in open-field test (mean ± SE)
Некоторые показатели поведения полевок Брандта в эксперименте открытого поля, $M \pm m$

Activity	Exposed animals	Control animals	Activity	Exposed animals	Control animals
Lat	8.40±2.15	3.30±1.12	Groom	0.60±0.17	1.30±0.30
Loco	90.70±9.16	132.00±10.84	Groomdur	11.00±5.02	6.80±1.83
Min1	25.10±2.98	37.20±2.88	Def	0.50±0.23	3.30±1.36
Min2	19.80±2.65	29.50±2.94	Urin	0.50±0.13	0.90±0.27
Min3	20.60±2.78	24.20±3.02	Immobile	36.90±10.35	2.20±1.51
Min4	13.40±1.93	24.60±13.67	Standsup	7.90±1.04	6.80±1.64
Min5	11.90±2.06	16.10±2.51	Standwsup	3.00±0.75	8.20±1.72
Firstcom	52.80±3.19	120.40±19.97	Tostand	11.50±1.23	17.90±1.16
N com	5.00±0.75	8.60±0.75	Dig	0.60±0.16	0.60±0.20
In box	0.90±0.24	1.60±0.35	Sneeze	1.45±0.28	0.60±0.20
On box	0.10±0.35	0.70±0.24	EA	0.60±0.13	1.40±0.13

cantly lower than that of control animals ($P<0.001$). The voles moved slower along the walls of the experimental box, with frequent stopping for 20–200 s, which is not usual for this species, so immobility of exposed animals showed statistically more often than in control voles ($P<0.001$). Sometimes immobility increased 15-fold or even to a greater extent, compared to that of control animals.

Experimental animals crossed the center of the field 22 times later, compared to that of control animals ($P<0.001$), 36.4% of them approached the food container located in the center of the field for the first time only after 2 min, three voles never approached it within 5 min observation.

The number of animals coming to the food container was also smaller in the experimental group than in the control one ($P<0.05$). The animals exposed to SMF entered the box and climbed on its roof less frequently ($P<0.05$). Vertical activity in the open field, i.e., standings on hind paws with or without support on the walls and box, is common in voles. Exposed animals demonstrated this type of activity not so often as the control ones ($P<0.001$). Vegetative reactions in the form of defecations were more frequent in control animals ($P<0.05$). The number of urinations was higher too, however the differences are not statistically significant.

The total index of exploratory activity expressed in points was lower in exposed animals ($P<0.001$). For instance, the EA of experimental voles was 0.6, as opposed to 1.38 in control voles.

INFLUENCE OF ORDER OF EXPERIMENT WITH SMF EFFECT

If SMF was applied prior to the first experiment, the locomotor activity of animals in the second experiment failed to show statistically significant variations, at the same time the animals crossed the center of the field and their exploratory activity increased sharply ($P<0.05$) from 0.33±0.16 to 1.40±0.22.

If the magnet was applied prior to the second experiment, the locomotor activity and exploratory activity diminished appreciably (1.33±0.16 and 0.67±0.15, $P<0.05$), the time of center crossing remaining the same.

The behavior of animals in two successive experiments without exposure to SMF was almost unchanged, the most significant difference being recorded in center-crossing time 68.00±19.70 and 27.20±5.92 s, respectively.

BEHAVIOR VARIABILITY THAT DEPENDS ON SMF VECTOR INDUCTION DIRECTION CHANGES, INFLUENCING ON VOLES

Analysis of data shows that SMF pole switching exerts a significant influence on many types of animal activity in open-field tests (Figure).

The overall locomotor activity of voles was diminished, the most noticeable inhibition of activity being caused by SS orientation of magnetic field (the average value 84.38, compared to 132.00 without magnet). The inhibition of locomotor activity was especially marked during the first and fourth minute of the experiment. In addition, in the first minute strong influence of SS vector (average value 20.75, without magnet 37.19), in the fourth – NN vector (average value 12.29, without magnet 24.75) was noted. A smaller number of groomings, urinations and defecations was recorded in response to SS and NN orientation with the same indexes.

NS orientation of the magnetic field most intensively affected the first center-crossing time and the total number of center-crossings; the first coming to the food container increased from 91.97 to 149.29 s, the number of center-crossings being decreased from 6.57 to 4.57, while the NS vector reduced the total number of standings and standings without support onto the walls of the open field.

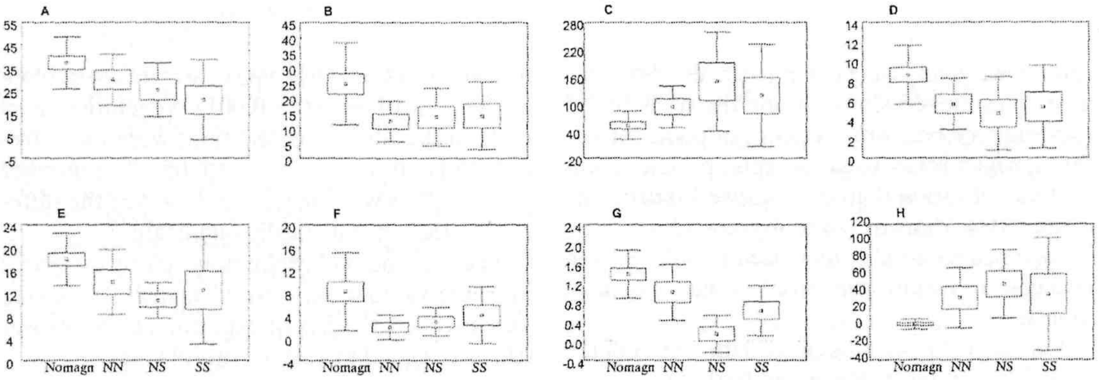

Fig. Statistically significant changes in behavioral parameters of Brandt's vole, which are dependent on SMF vector direction. A = first minute locomotor activity, Y-axis: number of squares crossed; B = fourth minute locomotor activity, Y-axis: numbers of squares crossed; C = first coming to the food container, Y-axis: time, s; D = number of comings to the food container; E = standing, Y-axis: number of standings; F = standing without support, Y-axis: number of standings; G = exploratory activity, Y-axis: index; H = immobility, Y-axis: time, s; X-axis: SMF applications. Standard deviation, standard error, mean value

Рис. Статистически значимые различия в поведении полевки Брандта при воздействии на них СМП: A – локомоторная активность в первую минуту эксперимента, на оси Y число пересеченных квадратов; B – локомоторная активность на четвертой минуте эксперимента, на оси Y число пересеченных квадратов; C – первое приближение к кормушке, на оси Y время, с; D – число приближений к кормушке; E – общее число стоек; F – число стоек без опоры; G – исследовательская активность, на оси Y индекс исследовательской активности; H – неподвижность, на оси Y время, с; на оси X показаны направления векторов СМП. Стандартное отклонение, стандартная ошибка, среднее значение

The effect of SMF on the total immobility index of animals was extremely manifest, the average value in the experimental group being 24.0 s, compared to 49.3 s in the case of NS orientation, which is 25 times in excess of the control value. The total immobility of animal safter exposure to SMF was increased to 33.1 s (NN orientation) and 37.5 s (SS orientation), respectively.

Different SMF orientation also affects such indexes as the number and duration of autogroomings, the number of climbings onto the box roof, diggings and general exploratory activity. NN vector decreased the number and duration of grooming in animals. Inhibition of the overall level of exploratory activity was noted in the case of NS orientation.

DISCUSSION

The behavior of experimental and control animals in the open-field test is similar and corresponds to the pattern described previously [14]. During the first stage of exploration of the open-field test area, active defensive behavioral patterns are characteristic and common in Brandt's voles [2]. The animals usually leave their box at once and start moving rapidly along the box walls of the open field, soon after the beginning of the experiment they cross the central part of the field. Besides diggings, gnawing, chisels' hits for the floor and box's walls are recorded.

In a stressful situation like this the active defensive reactions are more adaptive, because the stress caused by the factor of novelty can be overcome faster and the animals begin examining the new space at an earlier stage and demonstrate their exploratory behavior. At this stage vole's behavior is determined by the high level of emotional state (as evidenced by increased frequency of urinations and defecations). The active movements during the first minutes of the experiment represent one of the mechanisms of relaxation from emotional stress [8].

As soon as learning in the open field begins, the initial stress subsides and exploratory activity increases. Its beginning is marked by the increasing number of center-crosses and the number of vertical standings.

Exposure to magnetic field first of all results in a slower behavioral pattern, the animal leaves its temporary shelter at a later time and remains still more frequently and for a longer period of time near the walls of the open field. The emotional tension and stress seem to decrease in the animals, this causes inhibition of their exploratory activity and a reduced frequency of urinations and defecations. At the same time, this decreases the probability of transition to the learning phase in the new space, therefore the voles cross the center of the field less frequently and at a later time, they almost never examine the box and the food container, vertical activity is significantly less manifest, especially orientation standings without support. Immobility periods may be often connected with developing hyper defensive inhibition [5]. These authors believe that periods of relaxation are more clearly expressed in animals with a weaker nervous system. In our experiments this can be a result of magnetic field influence on the central nervous system of animals.

The results obtained indicate that SMF may affect excitation and inhibition processes in the CNS, thus decreasing the ability of animals to conform to the changing environment. Although control animals during a 5-min experiment are capable of forming an initial psychical image of the area and display a fairly good orientation in the new space, the animals exposed to magnetic field practically fail to demonstrate learning skills. Exposed voles show a much higher activity, which is aimed at finding a way out of the situation; they have a high level of standing with support onto the walls. At the same time, the real exploratory standings without support that are aimed at researching space are not expressed.

Changes in the order of SMF exposure in two experiments also confirm the inhibitory effect of SMF on the exploratory behavior of voles. The magnetic influence was noted in the first experiment, whereas in repeated experiments with the same animals only exploratory activity increased: the animals crossed the field faster and began to form spatial image of open field, all parameters of exploratory activity (number of standing, number of box-entrances, climbing upon it, etc.)

were increased. Unlike in the first experiment, when normal learning skills were noted, exposure to SMF in the second experiment caused a reverse change in all the parameters measured. In two experiments without magnetic influence the behavior of control animals did not differ, their space learning being almost on the same level or some diminished.

The assessment of the response to different poles' influence on animal behavior displayed different effect on different forms of animal behavior in an open field. NN and SS poles exerted a stronger effect on locomotor activity and emotionality (number of autogroomings, urinations and defecations). Immobility was influenced by all variants of poles. Opposite poles (NS) influenced stronger the exploratory activity, as expressed by center-crossing time, the number of through-approaches, standings, box-entrances and roof climbing, digging. It can be assumed that different parts in the animal brain are possibly responsible for exploratory activity and the ability to overcome stress in a novel situation, neofobia (fear of novelty).

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10. Kopsavilkums

Mūsu eksperimentos iegūtie dati par biogēno amīnu un to metabolītu summārām koncentrācijām žurkas smadzeņu audos atbilst citu laboratoriju datiem (Narita et al, 2002; Lowry et al., 2001) un apstiprina jau iegūtos novērojumus par atšķirībām starp dažādiem apvidiem un atšķirībām starp abu pusložu identiskiem apvidiem. Tā, pētījumā ar žurkām, parādīts dopamīna koncentrācijas pārsvars intaktiem dzīvniekiem kreisās puslodes svītrainā ķermeņa audos (Glick, Ross, 1981), kas apstiprinājās arī mūsu pētījumos: 10178±928 ng*kg^{-1} kreisajā puslodē pret 8810±928 ng*kg^{-1} labajā.

Literatūrā nav datu par PML ietekmi uz bioloģisko amīnu metabolismu smadzeņu audos. Pieejami vairāku laboratoriju pētījumu apraksti par ekstrēmi zemas frekvences (<300 Hz) magnētisku lauku iedarbību (WHO, 2006), taču datu salīdzināšanu apgrūtina atšķirības izmantoto lauku intensitātēs un aplikāciju īpatnībās. Piemēram, eksperimentos ar žurkām noskaidrojās, ka ekstrēmi zemas zemfrekvences lauks (1,8-3,8 mT, stundas ilga aplikācija 14 dienas) paaugstina DA un 5-HT sintēzi žurku pieres daivas garozā, bet samazina kopējo serotonīna koncentrāciju svītrainajā ķermenī, ietekmē arī monoamīnerģisko sistēmu reaktivitāti (Sieron et al., 2004)

Serotonīna sistēma izrādījās jutīga arī mūsu eksperimentos, aplicējot žurkas smadzenēm viīPML. Par serotonīna metabolisma jūtību pret viīPML liecina serotonīna koncentrācijas pieaugums somatosensorā garozā, hipotalāma svītrainajā ķermenī, samazināšanās jūras zirga audos un hidroksiindoletiķskābes/ serotonīna attiecības samazināšanās somatosensorā garozā, hipotalāmā, bet palielināšanās hipokampā.

Novēroto PML efektu skaidrojums, iespējams, vismaz daļēji saistāms ar bioķīmiķu atziņu, ka PML izmaina MAO konformāciju un aktivitāti, uz ko netieši norāda PML ietekme uz dažādiem enzīmiem (Young, 1969). 20mkT PML ietekmē novērots arī kalmodulin-atkarīgā ciklisko nukleotīdu fosfodiestērāzes aktivitātes pieaugums (Liboff et al., 2003).

Mūsu pētījums pārliecinoši demonstrē viīPML vektora orientācijas attiecībā pret smadzeņu struktūrām nozīmi. Izteiktākas 5-HT, 5-HIAA/5-HT (hipokampā un hipotalāmā), DA, DOPAC (striatumā un hipotalāmā) izmaiņas izraisīja viīPML, ja tas aplicēts ar indukcijas orientāciju lN-kN. Savukārt, 5-HT koncentrācijas izteikts pieaugums somatosensorā garozā un hipotalāmā viīPML ietekmē veidojās, ja tika aplicēts lauks ar indukcijas virzienu lS-kS. Šie novērojumi norāda uz vairāku smadzeņu apvidu īpašu jūtību pret vienādpolu viīPML aplikāciju abpus galvai. Diemžēl, vienādnosukuma polu aplikācijas biofizikālie aspekti nav izzināti, tāpēc arī mūsu eksperimentos novēroto efektu fizioloģiskajam skaidrojumam ir nepieciešami tālākie pētījumi. Līdzīgi, 5-HT koncentrācijas pieaugums PML ietekmē (intensitāte 50 mkT) tika novērots žurku epifīzē, un autori (Reiter, Richardson, 1992) izvirzīja pieņēmumu, ka magnētiskais lauks izraisa enzīma N-acetīltransferāzes inhibiciju, kā rezultātā pieaug arī 5-HIAA koncentrācija un samazinās melatonīna produkcija epifīzē.

Mūsu veiktajos eksperimentos noskaidrojās, ka viīPML (neatkarīgi no lauka polaritātes) spēj izraisīt somatosensoro izsaukto potenciālu latento periodu izmaiņas, kā likums – latentā perioda pagarināšanos. Taču lauka ietekmes efektivitāte ir atkarīga no lauka orientācijas pret smadzenēm. Līdzīgi novērojumi ir aprakstīti citu autoru pētījumos, piemērām, ir parādīts, ka zemas intensitātes (34mT) PML ar neilgu aplikācijas laiku (20 minūtes) izraisa nervu impulsa izplatīšanās ātruma samazināšanos: dati iegūti eksperimentos ar vardes gūžas nervu - muskuļu preparātu (Овчинников, 1994).

viīPML vektora orientācijas pret smadzenēm nozīmes izvērtēšanai tika veltīti pētījumi sadaļā par viīPML ietekmi uz žurkas autonomo funkciju vadības mehānismiem. Sirds ritma vadības simpātiskie un parasimpātiskie centri veido nepāra daudzkomponentu struktūru galvas smadzeņu hipotalāma un stumbra daļās. viīPML tika aplicēts, novietojot magnētus abpus dzīvnieka galvai; līdz ar to, homogēna lauka ietekmes zonā ietilpa visas galvas smadzenes, bet lauka perifērija, iespējams, aptvēra arī muguras smadzeņu un simpātiskā stumbra augšdaļu.

Lielākai daļai (81%) dzīvnieku ilgstošā EKG pierakstā labi saskatāmi cikla ilguma viļņi ar tādu viļņa garumu, pie kura katrā vilnī ietilpst aptuveni četri secīgi cikli. To izcelsme skaidrojama ar elpošanas centra ietekmi uz sirdsdarbības centru un izpaužas kā respiratora sinusa aritmija (RSA).

Mūsu eksperimentu rezultāti parāda, ka lielai daļai, bet ne visiem dzīvniekiem, bija vērojama izteikta RSA, tātad salīdzinoši augsta parasimpātiskās aktivitātes dominante hipotalāma un medullas struktūrās. Toties viīPML (neatkarīgi no tā orientācijas pret dzīvnieka galvu) šo parasimpātisko dominanti nomāca. Mūsu eksperimenti parādīja arī to, ka dzīvnieka reakcijai uz viīPML ir izteikti individuālas atšķirības; viens no to cēloņiem varētu būt atšķirīgas simpātisko un parasimpātisko mehānismu aktivitātes. Zīmīgi, ka dzīvniekiem, kuriem fona apstākļos nebija vērojama izteikta sinusa aritmija (bija salīdzinoši augsta simpātiska aktivitāte), viīPML aplikācija izraisīja noturīgu sirdsdarbības frekvences pazemināšanos.

Eksperimenti paradīja, ka viīPML ietekme uz eksperimenta dzīvniekiem visspilgtāk izpaudās divos fenomenos - sirdsdarbības palēnināšanās un elpošanas aritmijas izzušana. Kā hipotēzi var izvirzīt pieņēmumu, ka viīPML neatkarīgi ietekmē divas atšķirīgas autonomas nervu sistēmas struktūras: pirmkārt, nomāc hipotalāma sirdsdarbības paātrinošo centru un, otrkārt, nomāc stumbra neironus, kas reciproki saista elpošanas centru un sirds ritma neirālās vadības centru. Rezultāti ļauj secināt, ka viīPML efektivitāte ir atkarīga no abu šo centru toniskās aktivitātes līmeņa. To pierāda novērojums, ka visiem četriem dzīvniekiem ar salīdzinoši zemu stumbra parasimpatisko centru jūtību (pirms viīPML ietekmes nebija vērojama elpošanas aritmija) viīPML aplikācija izraisīja sirds darbības ritma paātrināšanos, nevis palēnināšanos.

Fizioloģisko funkciju autonomās vadības centru jutība pret PML pierādījusies vairāku autoru pētījumos. Piemēram, trušiem tika novērota šo dzīvnieku izteikta jutība pret ģeomagnētiskā lauka izmaiņām, kas izpaudās gan sirdsdarbības frekvences, gan arteriālā spiediena, gan citos autonomos vadības mehānismos. Pētījumu autori nonāca pie secinājuma, ka Zemes magnētiskā lauka dabiskās nelielās svārstības spēj ietekmēt gan smadzeņu stumbra, gan augstākos sirds un asinsvadu regulācijas centrus (Gmitrov, Gmitrova, 2004). Līdzīgi secinājumi izriet arī no pētījumiem par ģeomagnētiskās vētras ietekmi uz cilvēka asinsrites regulācijas mehānismiem (Dmitrova et al., 2004). Dati par PML (un arī elektromagnētisko lauku) ietekmi tieši uz sirdsdarbības centrālās vadības mehānismiem ir pretrunīgi un grūti salīdzināmi. Pētījumos ar cilvēkiem daži autori apstiprina mainīgā lauka (60Hz) ietekmi uz sirdsdarbības frekvenci (Griefahn et al., 2001; Sastre et al., 1998), citi šādus efektus neapstiprina (Graham et al., 2000; Okano, Ohkubo, 2005).

Minētie rezultāti kopumā ļauj izvirzīt pieņēmumu, ka PML ietekmes uz autonomo funkciju vadību var būtiski atšķirties dažādos lauka intensitātes diapazonos, kā arī būt atkarīgas no dzīvnieka sugas un lauka aplikācijas īpatnībām.

Jautājums par smadzeņu psihosomātisko funkciju vadības struktūru jutību pret ārējo magnetisko lauku ietekmēm uzskatāms par aktuālu sakarā ar tehnisku ierīču, kas ģenerē elektromagnētisku lauku, izplatību. Līdz ar to, ar katru gadu palielinās pētījumu skaits par dažādas frekvences un intensitātes elektromagnētisko lauku bioloģiskiem efektiem, taču lielākai daļai šo pētījumu ir lietišķa rakstura mērķi – pamatot darba drošības kritērijus, strādājot ar šīm ierīcēm (Wilen et al., 2004; Kheifets et al., 2006). Praktiski iztrūkst neirofizioloģiska vai etoloģiska rakstura pētījumi par PML iespējamo ietekmju fizioloģiskajiem mehānismiem. Līdz ar to, apgrūtināts ir mūsu pētījumos par PML ietekmi uz dzīvnieku instinktīvu uzvedību iegūto rezultātu salīdzinošs izvērtējums.

Iegūtie rezultāti liecina, ka vairumā gadījumu viīPML ietekmei ir uzvedības aktivitāti mazinošs raksturs. Bez tam, noskaidrojās, ka dažādiem lauka vektoru virzieniem ir atšķirīga efektivitāte. Visefektīvākie izrādījās viennosaukuma polu lauki NN un SS, pie kam visjutīgākie uzvedības parametri ir summārā lokomotorā aktivitāte un emocionālas uzvedības parametri (kasīšanās, urinācijas un defekācijas skaits laika vienībā). Toties pretpolu viīPML aplikācija vairāk iespaidoja izpētes stadiju (centra šķērsošanas skaits, vertikālā aktivitāte, mājiņu izpēte, ostīšana).

Literatūrā aprakstītos pētījumos par zemfrekvences (<300Hz) elektromagnētiskā lauka iedarbību uz pelēm tika novērots, ka, līdzīgi kā mūsu eksperimentos, lauka ietekmē samazinājās izpētes aktivitātes laiks un vertikāla aktivitāte, pieauga miegā pavadītais laiks (Del Seppia et al., 2003).

No veikto eksperimentu metodoloģijas viedokļa par būtisku, mūsuprāt, uzskatāms individuālo rezultātu neviendabīgums (variabilitāte). Tas netieši norāda uz to, ka PML raksturo vairāki neatkarīgi parametri (stiprums, virziens, aplikācijas ilgums), kuri katrs par sevi var būt bioloģiski nozīmīgi un ar savu specifisku ietekmi. Šīs ietekmes, visdrīzāk, nav viennozīmīgas, bet gan atkarīgas no indivīda īpatnībām un bioloģiskās struktūras (smadzeņu kopumā, atsevišķu centru, sinapšu) funkcionālā stāvokļa viīPML aplikācijas laikā.

Iegūtie rezultāti kopumā ļauj izvirzīt hipotēzi, ka viīPML indukcijas vektora virzienam attiecībā pret organisma anatomiskām struktūrām izteiktāka loma ir tajos gadījumos, ja lauka ietekmei ir pakļautas pāra struktūras (piemēram, galvas smadzeņu garozas lielās puslodes, vidussmadzeņu struktūras), kamēr nepāra struktūru gadījumā (piemēram, hipotalāms un stumbra kardio-vaskulāras vadības centri) viīPML orientācijas loma ir mazāk nozīmīga.

11. Secinājumi

1. Mākslīgs vidējas intensitātes īslaicīgi aplicēts pastāvīgais magnētiskais lauks (viīPML; intensitāte 250 mT, aplikācijas ilgums 15 minūtes), kas aplicēts laboratorijas dzīvnieka ķermenim vai galvai, izraisa plaša spektra tūlītējas funkcionālas izmaiņas visā organismā, t.sk., ietekmē monoamīnu koncentrāciju smadzenēs, smadzeņu vadības un psihiskās funkcijas, veģetatīvo funkciju regulācijas mehānismus un dzīvnieku uzvedību.

2. Būtisks viīPML parametrs, no kura atkarīga gan lauka efektivitāte, gan fizioloģiskās reakcijas kvalitatīvās izpausmes, ir magnētiskā lauka vektora orientācija pret dzīvnieka ķermeņa un smadzeņu anatomiskām struktūrām. Pirmo reizi izdevies parādīt smadzeņu audu īpašu jūtību pret PML viennosaukuma polu aplikāciju abpus galvai.

3. Viena no viīPML ietekmes uz smadzenēm izpausmēm ir psihosomatisko procesu kavēšana, par ko liecina somatosensoro izsaukto potenciālu latento periodu pagarināšanās, elektrokortikogrammas zemas frekvences viļņu amplitūdas palielināšanās, instinktīvo uzvedības reakciju gausināšanās.

4. PML īslaicīga (15 minūtes) iedarbība uz narkotizēto žurku galvas smadzenēm ietekmē sirds darbības autonomās regulācijas augstākos centrus, mainot simpātisko un parasimpātisko ietekmju līdzsvaru.

5. PML īslaicīga (15 minūtes) iedarbība uz narkotizēto žurku galvas smadzenēm izraisa statistiski ticamas monoamīnu koncentrācijas izmaiņas smadzeņu audos, kas ir atšķirīgas dažādos smadzeņu apvidos (pieres daļas garoza, svītrainais ķermenis, hipotalāms un hipokamps) un atkarīgas no lauka vektora orientācijas pret smadzenēm. Paaugstināta bioķīmisko procesu jūtība tika konstatēta viennosaukuma polu magnētu aplikāciju gadījumos.

6. Akūtos un hroniskos eksperimentos ar žurkām un trušiem, kuros tika vērtēti somatosensorās vadības funkcionālie parametri (izsaukto potenciālu latentais periods, elektrokortikogrammu dominējošās frekvences un viļņu amplitūdas), iegūtie rezultāti norāda vienas noteiktas vektoru orientācijas (abpus smadzenēm Ziemeļu pols – eksperimentos ar žurkām, vai truša smadzeņu garozā iedzīvinātais elektrods, kas magnetizēts kā Ziemeļu pols) selektīvi augsto efektivitāti, salīdzinot ar citām lauka orientācijām.

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За мгновеньем мгновенье - и жизнь промелькнет.
Пусть весельем мгновение это блеснет!
Берегись, ибо жизнь - это сущность творенья,
Как ее проведешь, так она и пройдет.
Хайям

Pielikums Nr.1

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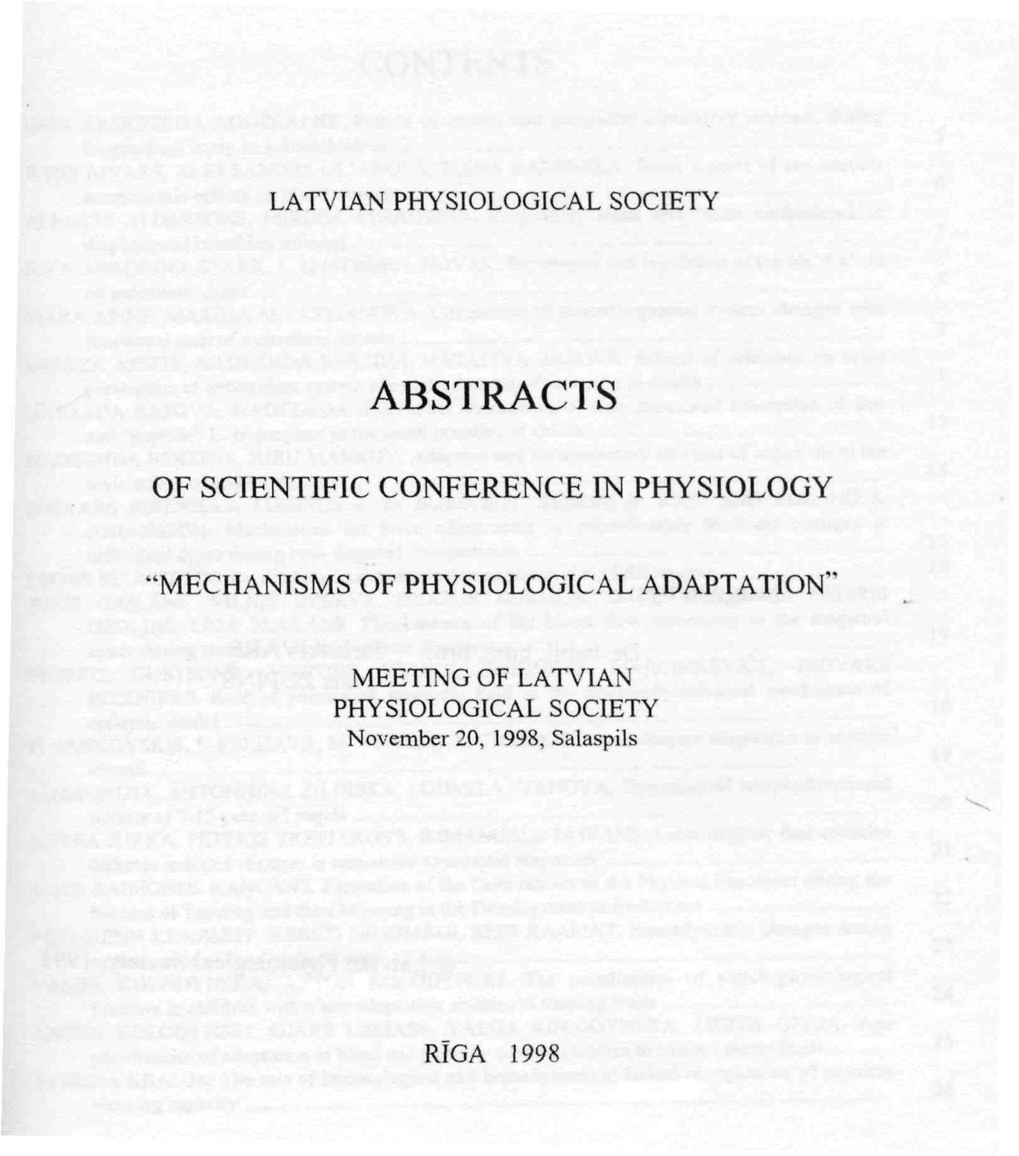

LATVIAN PHYSIOLOGICAL SOCIETY

ABSTRACTS

OF SCIENTIFIC CONFERENCE IN PHYSIOLOGY

"MECHANISMS OF PHYSIOLOGICAL ADAPTATION"

MEETING OF LATVIAN
PHYSIOLOGICAL SOCIETY
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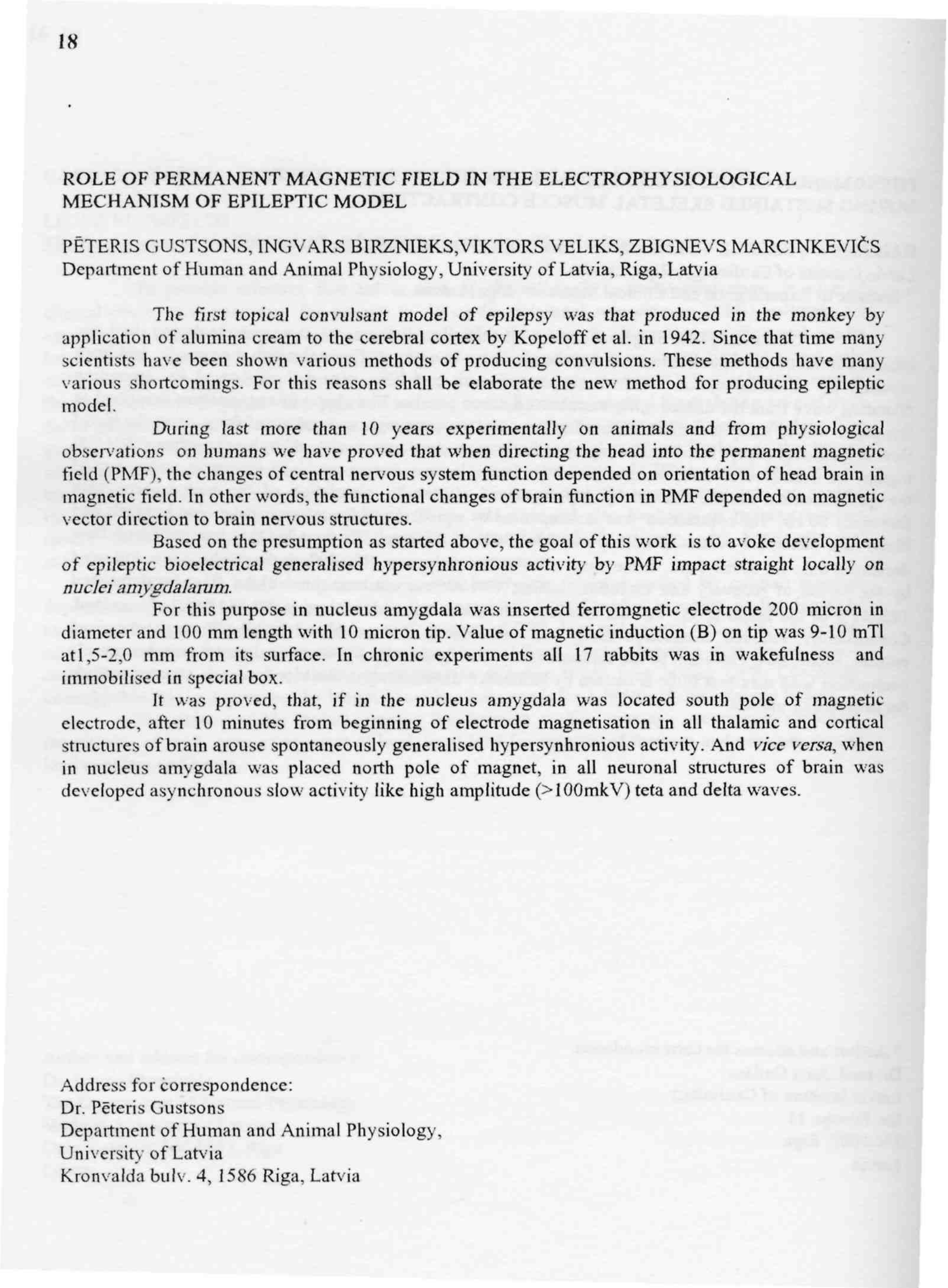
RĪGA 1998

- V.Veliks, Z.Marcinkevičs, P.Gustsons, I.Birznieks. Influence of permanent magnetic field on impulse propagation in central nervous system.



ROLE OF PERMANENT MAGNETIC FIELD IN THE ELECTROPHYSIOLOGICAL MECHANISM OF EPILEPTIC MODEL

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The first topical convulsant model of epilepsy was that produced in the monkey by application of alumina cream to the cerebral cortex by Kopeloff et al. in 1942. Since that time many scientists have been shown various methods of producing convulsions. These methods have many various shortcomings. For this reasons shall be elaborate the new method for producing epileptic model.

During last more than 10 years experimentally on animals and from physiological observations on humans we have proved that when directing the head into the permanent magnetic field (PMF), the changes of central nervous system function depended on orientation of head brain in magnetic field. In other words, the functional changes of brain function in PMF depended on magnetic vector direction to brain nervous structures.

Based on the presumption as started above, the goal of this work is to avoke development of epileptic bioelectrical generalised hypersynhronious activity by PMF impact straight locally on *nuclei amygdalarum.*

For this purpose in nucleus amygdala was inserted ferromgnetic electrode 200 micron in diameter and 100 mm length with 10 micron tip. Value of magnetic induction (B) on tip was 9-10 mTl at1,5-2,0 mm from its surface. In chronic experiments all 17 rabbits was in wakefulness and immobilised in special box.

It was proved, that, if in the nucleus amygdala was located south pole of magnetic electrode, after 10 minutes from beginning of electrode magnetisation in all thalamic and cortical structures of brain arouse spontaneously generalised hypersynhronious activity. And *vice versa*, when in nucleus amygdala was placed north pole of magnet, in all neuronal structures of brain was developed asynchronous slow activity like high amplitude (>100mkV) teta and delta waves.

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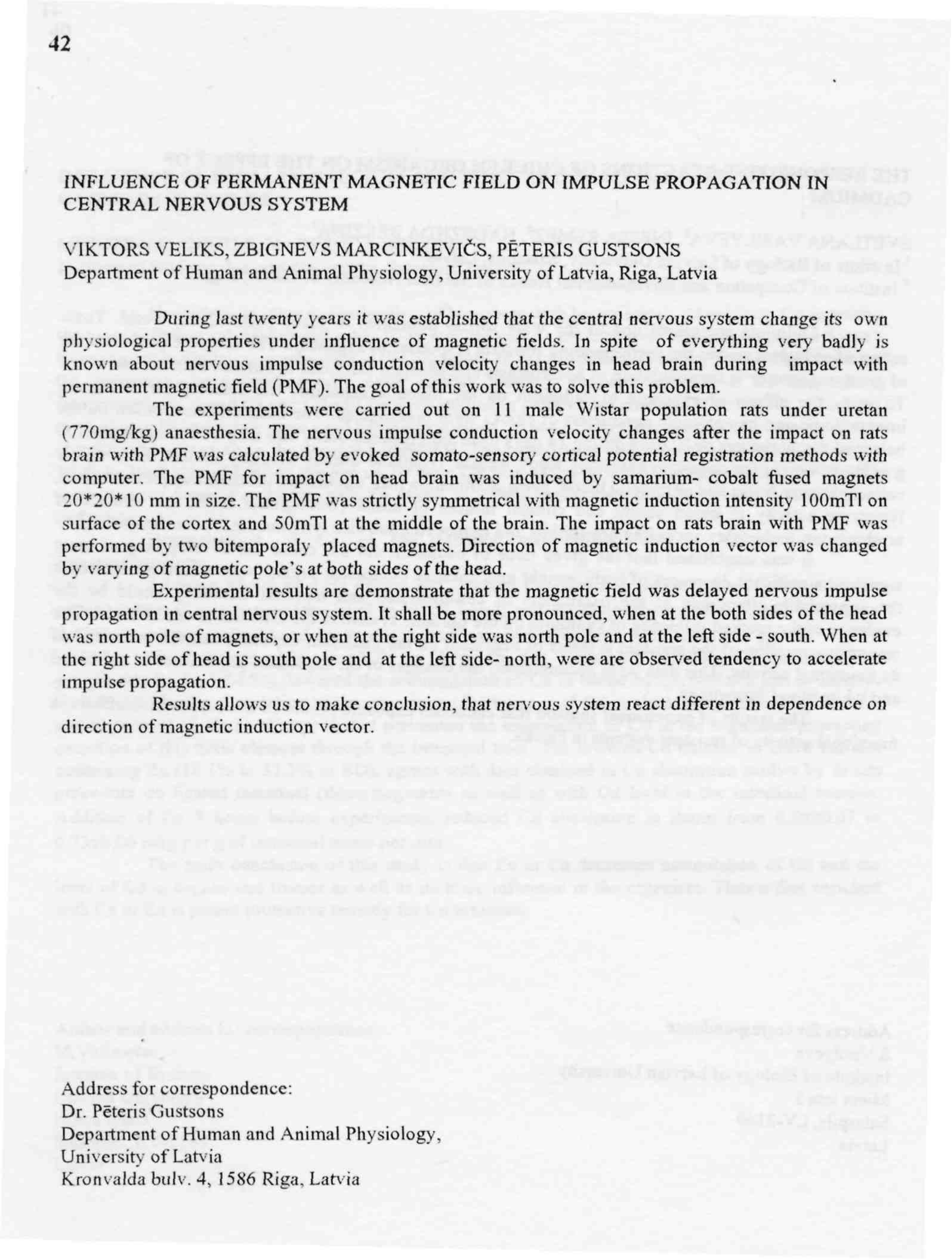
- P.Gustsons, V.Veliks, Z.Marcinkevičs. I.Birznieks. Role of permanent magnetic field in the electrophysiological mechanism of epileptic model.



INFLUENCE OF PERMANENT MAGNETIC FIELD ON IMPULSE PROPAGATION IN CENTRAL NERVOUS SYSTEM

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During last twenty years it was established that the central nervous system change its own physiological properties under influence of magnetic fields. In spite of everything very badly is known about nervous impulse conduction velocity changes in head brain during impact with permanent magnetic field (PMF). The goal of this work was to solve this problem.

The experiments were carried out on 11 male Wistar population rats under uretan (770mg/kg) anaesthesia. The nervous impulse conduction velocity changes after the impact on rats brain with PMF was calculated by evoked somato-sensory cortical potential registration methods with computer. The PMF for impact on head brain was induced by samarium- cobalt fused magnets 20*20*10 mm in size. The PMF was strictly symmetrical with magnetic induction intensity 100mTl on surface of the cortex and 50mTl at the middle of the brain. The impact on rats brain with PMF was performed by two bitemporaly placed magnets. Direction of magnetic induction vector was changed by varying of magnetic pole's at both sides of the head.

Experimental results are demonstrate that the magnetic field was delayed nervous impulse propagation in central nervous system. It shall be more pronounced, when at the both sides of the head was north pole of magnets, or when at the right side was north pole and at the left side - south. When at the right side of head is south pole and at the left side- north, were are observed tendency to accelerate impulse propagation.

Results allows us to make conclusion, that nervous system react different in dependence on direction of magnetic induction vector.

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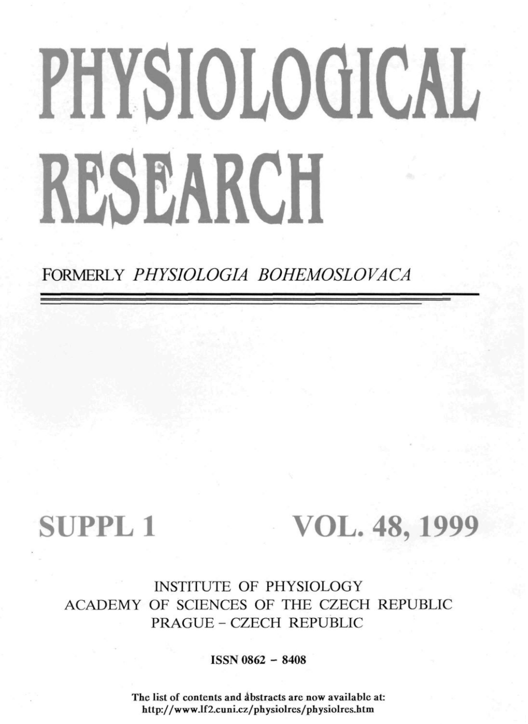

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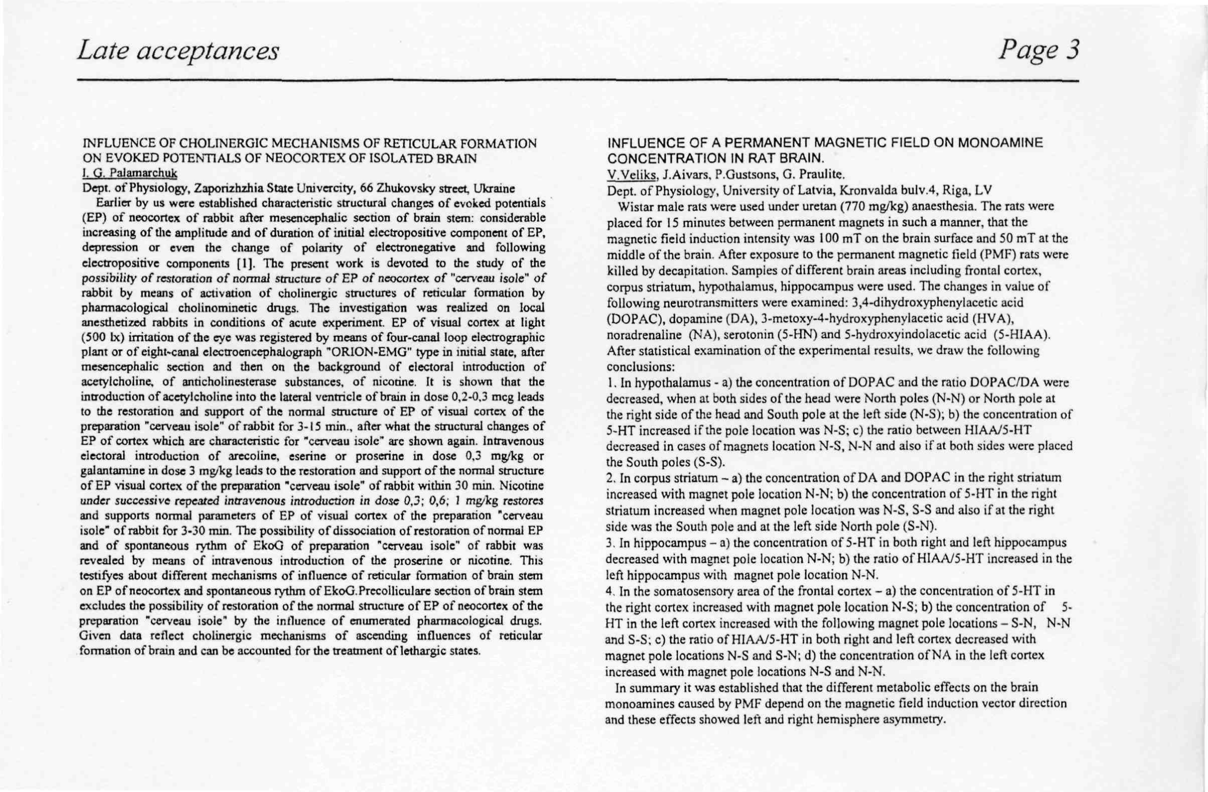
- V.Veliks, J.Aivars, P.Gustsons, G. Praulite. Influence of a permanent magnetic field on monoamine concentration in rat brain. Physiological research Suppl.1., Vol. 48, 1999 Prague p.3.



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INFLUENCE OF CHOLINERGIC MECHANISMS OF RETICULAR FORMATION ON EVOKED POTENTIALS OF NEOCORTEX OF ISOLATED BRAIN

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Earlier by us were established characteristic structural changes of evoked potentials (EP) of neocortex of rabbit after mesencephalic section of brain stem: considerable increasing of the amplitude and of duration of initial electropositive component of EP, depression or even the change of polarity of electronegative and following electropositive components [1]. The present work is devoted to the study of the *possibility of restoration of normal structure of EP of neocortex of "cerveau isole" of* rabbit by means of activation of cholinergic structures of reticular formation by pharmacological cholinominetic drugs. The investigation was realized on local anesthetized rabbits in conditions of acute experiment. EP of visual cortex at light (500 lx) irritation of the eye was registered by means of four-canal loop electrographic plant or of eight-canal electroencephalograph "ORION-EMG" type in initial state, after mesencephalic section and then on the background of electoral introduction of acetylcholine, of anticholinesterase substances, of nicotine. It is shown that the introduction of acetylcholine into the lateral ventricle of brain in dose 0,2-0,3 mcg leads to the restoration and support of the normal structure of EP of visual cortex of the preparation "cerveau isole" of rabbit for 3-15 min., after what the structural changes of EP of cortex which are characteristic for "cerveau isole" are shown again. Intravenous electoral introduction of arecoline, eserine or proserine in dose 0,3 mg/kg or galantamine in dose 3 mg/kg leads to the restoration and support of the normal structure of EP visual cortex of the preparation "cerveau isole" of rabbit within 30 min. Nicotine *under successive repeated intravenous introduction in dose 0,3; 0,6; 1 mg/kg restores* and supports normal parameters of EP of visual cortex of the preparation "cerveau isole" of rabbit for 3-30 min. The possibility of dissociation of restoration of normal EP and of spontaneous rythm of EkoG of preparation "cerveau isole" of rabbit was revealed by means of intravenous introduction of the proserine or nicotine. This testifyes about different mechanisms of influence of reticular formation of brain stem on EP of neocortex and spontaneous rythm of EkoG.Precolliculare section of brain stem excludes the possibility of restoration of the normal structure of EP of neocortex of the preparation "cerveau isole" by the influence of enumerated pharmacological drugs. Given data reflect cholinergic mechanisms of ascending influences of reticular formation of brain and can be accounted for the treatment of lethargic states.

INFLUENCE OF A PERMANENT MAGNETIC FIELD ON MONOAMINE CONCENTRATION IN RAT BRAIN.

V.Veliks, J.Aivars, P.Gustsons, G. Praulite.

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Wistar male rats were used under uretan (770 mg/kg) anaesthesia. The rats were placed for 15 minutes between permanent magnets in such a manner, that the magnetic field induction intensity was 100 mT on the brain surface and 50 mT at the middle of the brain. After exposure to the permanent magnetic field (PMF) rats were killed by decapitation. Samples of different brain areas including frontal cortex, corpus striatum, hypothalamus, hippocampus were used. The changes in value of following neurotransmitters were examined: 3,4-dihydroxyphenylacetic acid (DOPAC), dopamine (DA), 3-metoxy-4-hydroxyphenylacetic acid (HVA), noradrenaline (NA), serotonin (5-HN) and 5-hydroxyindolacetic acid (5-HIAA). After statistical examination of the experimental results, we draw the following conclusions:

1. In hypothalamus - a) the concentration of DOPAC and the ratio DOPAC/DA were decreased, when at both sides of the head were North poles (N-N) or North pole at the right side of the head and South pole at the left side (N-S); b) the concentration of 5-HT increased if the pole location was N-S; c) the ratio between HIAA/5-HT decreased in cases of magnets location N-S, N-N and also if at both sides were placed the South poles (S-S).
2. In corpus striatum – a) the concentration of DA and DOPAC in the right striatum increased with magnet pole location N-N; b) the concentration of 5-HT in the right striatum increased when magnet pole location was N-S, S-S and also if at the right side was the South pole and at the left side North pole (S-N).
3. In hippocampus – a) the concentration of 5-HT in both right and left hippocampus decreased with magnet pole location N-N; b) the ratio of HIAA/5-HT increased in the left hippocampus with magnet pole location N-N.
4. In the somatosensory area of the frontal cortex – a) the concentration of 5-HT in the right cortex increased with magnet pole location N-S; b) the concentration of 5-HT in the left cortex increased with the following magnet pole locations – S-N, N-N and S-S; c) the ratio of HIAA/5-HT in both right and left cortex decreased with magnet pole locations N-S and S-N; d) the concentration of NA in the left cortex increased with magnet pole locations N-S and N-N.

In summary it was established that the different metabolic effects on the brain monoamines caused by PMF depend on the magnetic field induction vector direction and these effects showed left and right hemisphere asymmetry.

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в БИОЛОГИИ и МЕДИЦИНЕ

ТЕЗИСЫ

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ABSTRACTS

WEAK and HYPERWEAK FIELDS and RADIATIONS
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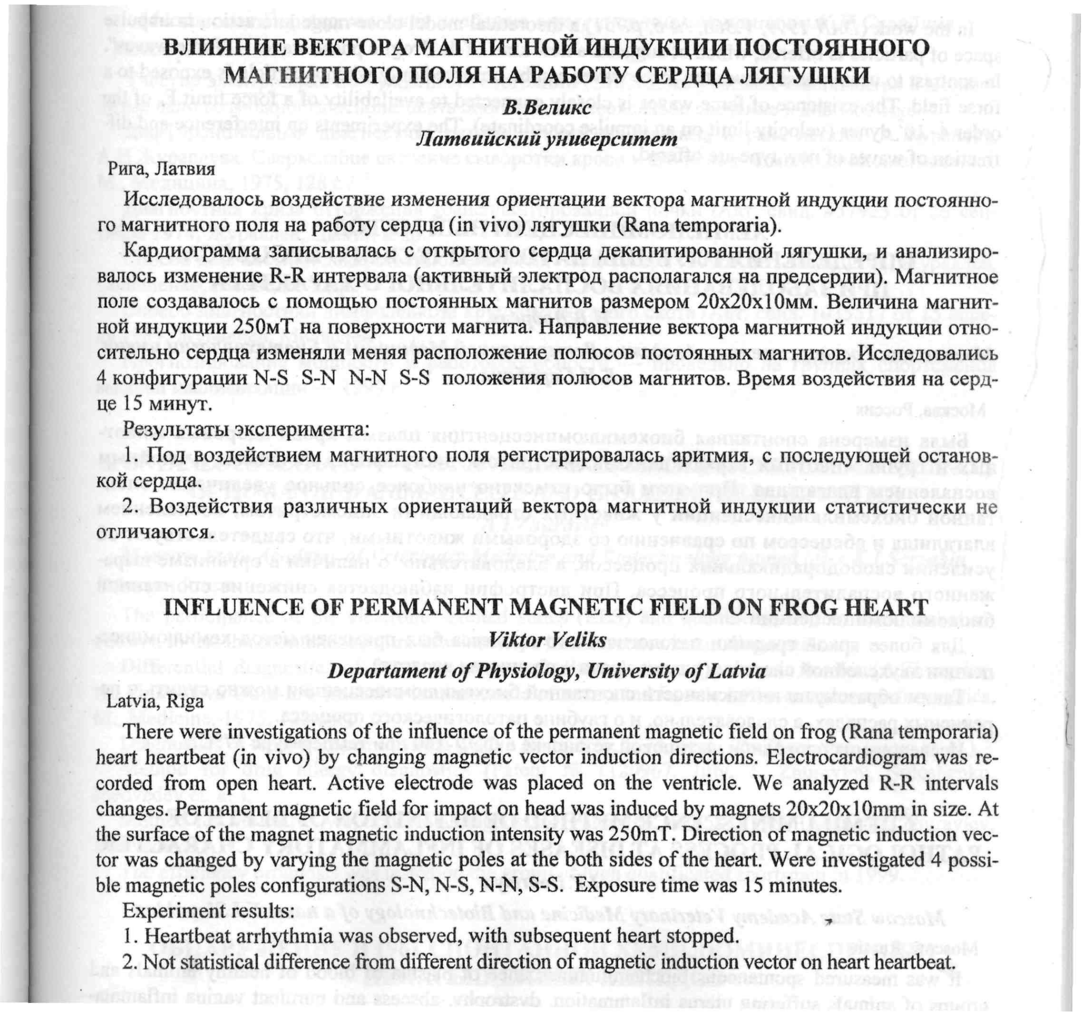
- V.Veliks. Influence of permanent magnetic field magnetic field on frog heart. II International Congress. Weak and hyperweak fields and radiations in biology and medicine. Sankt-Peterburg 2000.



ВЛИЯНИЕ ВЕКТОРА МАГНИТНОЙ ИНДУКЦИИ ПОСТОЯННОГО МАГНИТНОГО ПОЛЯ НА РАБОТУ СЕРДЦА ЛЯГУШКИ

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Исследовалось воздействие изменения ориентации вектора магнитной индукции постоянного магнитного поля на работу сердца (in vivo) лягушки (Rana temporaria).

Кардиограмма записывалась с открытого сердца декапитированной лягушки, и анализировалось изменение R-R интервала (активный электрод располагался на предсердии). Магнитное поле создавалось с помощью постоянных магнитов размером 20x20x10мм. Величина магнитной индукции 250мТ на поверхности магнита. Направление вектора магнитной индукции относительно сердца изменяли меняя расположение полюсов постоянных магнитов. Исследовались 4 конфигурации N-S S-N N-N S-S положения полюсов магнитов. Время воздействия на сердце 15 минут.

Результаты эксперимента:

1. Под воздействием магнитного поля регистрировалась аритмия, с последующей остановкой сердца.
2. Воздействия различных ориентаций вектора магнитной индукции статистически не отличаются.

INFLUENCE OF PERMANENT MAGNETIC FIELD ON FROG HEART

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There were investigations of the influence of the permanent magnetic field on frog (Rana temporaria) heart heartbeat (in vivo) by changing magnetic vector induction directions. Electrocardiogram was recorded from open heart. Active electrode was placed on the ventricle. We analyzed R-R intervals changes. Permanent magnetic field for impact on head was induced by magnets 20x20x10mm in size. At the surface of the magnet magnetic induction intensity was 250mT. Direction of magnetic induction vector was changed by varying the magnetic poles at the both sides of the heart. Were investigated 4 possible magnetic poles configurations S-N, N-S, N-N, S-S. Exposure time was 15 minutes.

Experiment results:

1. Heartbeat arrhythmia was observed, with subsequent heart stopped.
2. Not statistical difference from different direction of magnetic induction vector on heart heartbeat.

Fiziologu XXXIV starptautiskais kongress. Kraistčērčā. Jaunzelandē. 26.-31.07.2001.

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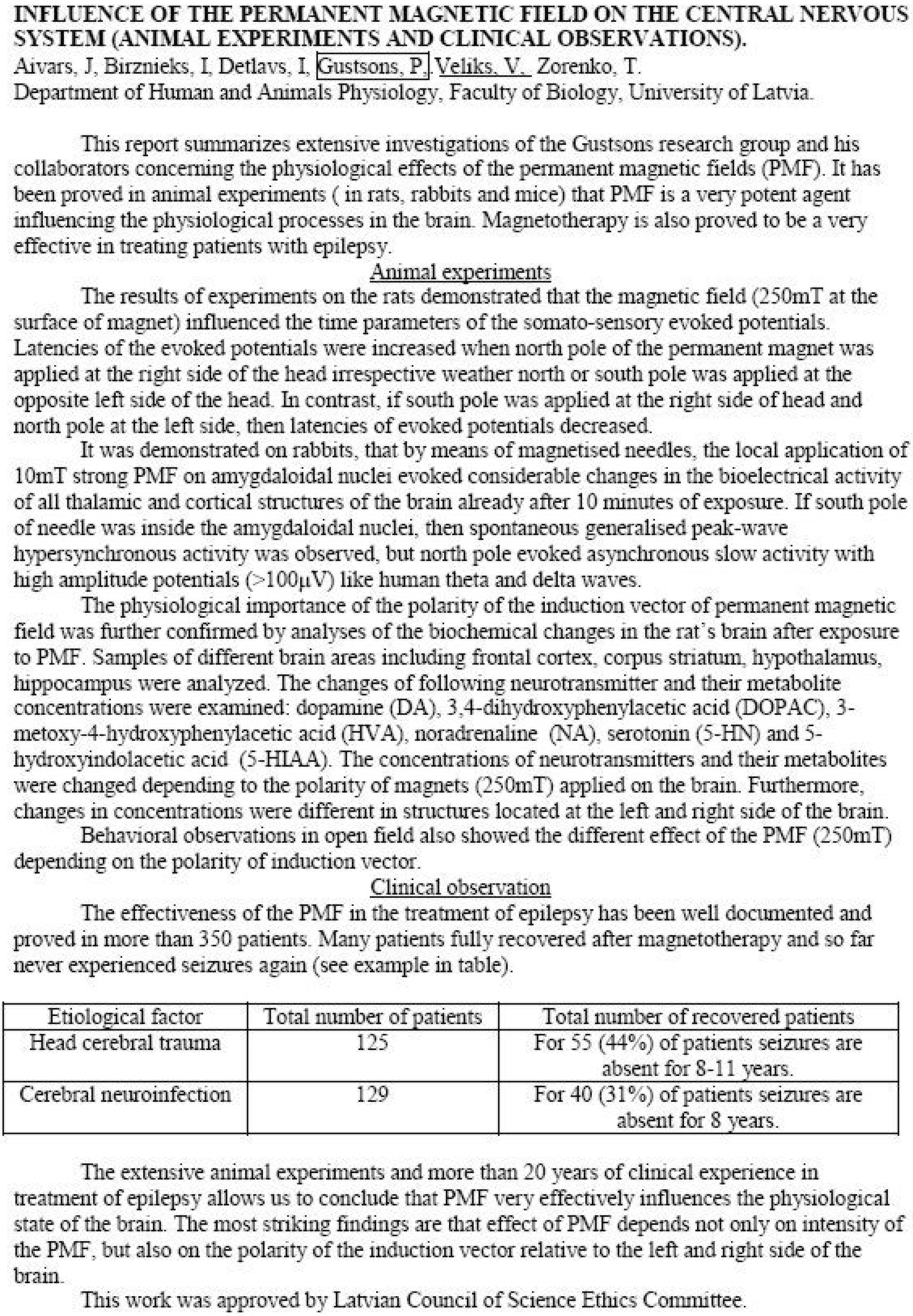
- V.Veliks, J.Aivars, P.Gustsons, I.Detlavs, I.Birznieks, T.Zorenko. Influence of the Permanent Magnetic Field on the Central Nervous System (Animal Experiments and Clinical Observations). Christchurch. New Zeland. 2001.



INFLUENCE OF THE PERMANENT MAGNETIC FIELD ON THE CENTRAL NERVOUS SYSTEM (ANIMAL EXPERIMENTS AND CLINICAL OBSERVATIONS).

Aivars, J, Birznieks, I, Detlavs, I, Gustsons, P, Veliks, V, Zorenko, T.
Department of Human and Animals Physiology, Faculty of Biology, University of Latvia.

This report summarizes extensive investigations of the Gustsons research group and his collaborators concerning the physiological effects of the permanent magnetic fields (PMF). It has been proved in animal experiments (in rats, rabbits and mice) that PMF is a very potent agent influencing the physiological processes in the brain. Magnetotherapy is also proved to be a very effective in treating patients with epilepsy.

Animal experiments

The results of experiments on the rats demonstrated that the magnetic field (250mT at the surface of magnet) influenced the time parameters of the somato-sensory evoked potentials. Latencies of the evoked potentials were increased when north pole of the permanent magnet was applied at the right side of the head irrespective weather north or south pole was applied at the opposite left side of the head. In contrast, if south pole was applied at the right side of head and north pole at the left side, then latencies of evoked potentials decreased.

It was demonstrated on rabbits, that by means of magnetised needles, the local application of 10mT strong PMF on amygdaloidal nuclei evoked considerable changes in the bioelectrical activity of all thalamic and cortical structures of the brain already after 10 minutes of exposure. If south pole of needle was inside the amygdaloidal nuclei, then spontaneous generalised peak-wave hypersynchronous activity was observed, but north pole evoked asynchronous slow activity with high amplitude potentials (>100μV) like human theta and delta waves.

The physiological importance of the polarity of the induction vector of permanent magnetic field was further confirmed by analyses of the biochemical changes in the rat's brain after exposure to PMF. Samples of different brain areas including frontal cortex, corpus striatum, hypothalamus, hippocampus were analyzed. The changes of following neurotransmitter and their metabolite concentrations were examined: dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 3-metoxy-4-hydroxyphenylacetic acid (HVA), noradrenaline (NA), serotonin (5-HN) and 5-hydroxyindolacetic acid (5-HIAA). The concentrations of neurotransmitters and their metabolites were changed depending to the polarity of magnets (250mT) applied on the brain. Furthermore, changes in concentrations were different in structures located at the left and right side of the brain.

Behavioral observations in open field also showed the different effect of the PMF (250mT) depending on the polarity of induction vector.

Clinical observation

The effectiveness of the PMF in the treatment of epilepsy has been well documented and proved in more than 350 patients. Many patients fully recovered after magnetotherapy and so far never experienced seizures again (see example in table).

Etiological factor	Total number of patients	Total number of recovered patients
Head cerebral trauma	125	For 55 (44%) of patients seizures are absent for 8-11 years.
Cerebral neuroinfection	129	For 40 (31%) of patients seizures are absent for 8 years.

The extensive animal experiments and more than 20 years of clinical experience in treatment of epilepsy allows us to conclude that PMF very effectively influences the physiological state of the brain. The most striking findings are that effect of PMF depends not only on intensity of the PMF, but also on the polarity of the induction vector relative to the left and right side of the brain.

This work was approved by Latvian Council of Science Ethics Committee.