Институт эволюционной физиологии и биохимии
им. И.М. Сеченова РАН

RUSNEUROCHEM

КОНФЕРЕНЦИЯ РОССИЙСКОГО НЕЙРОХИМИЧЕСКОГО ОБЩЕСТВА

22-24 МАЯ 2022

СБОРНИК ТЕЗИСОВ

г. Санкт-Петербург

Министерство науки и высшего образования Российской Федерации

Федеральное государственное бюджетное учреждение науки
Институт эволюционной физиологии и биохимии им. И.М. Сеченова РАН

Научный центр мирового уровня Павловский центр
«Интегративная физиология – медицине, высокотехнологичному здравоохранению и технологиям стрессоустойчивости»

RUSNEUROCHEM 2022

Всероссийская с международным участием конференция
Российского нейрохимического общества
22-24 мая 2022 года

ТЕЗИСЫ ДОКЛАДОВ

Санкт-Петербург
2022

УДК 577.11:577.25:615.9

Рецензент: академик РАН, д.б.н., зав. лаб. клеточной нейробиологии обучения ИВНД и НФ Балабан П.М.

RUSNEUROCHEM 2022: Всероссийская с международным участием конференция Российского нейрохимического общества, г. Санкт-Петербург (22-24 мая 2022 г.). – Тезисы докладов. – СПб.: Институт эволюционной физиологии и биохимии им. И.М.Сеченова РАН, 2022. 188 с.

В настоящем издании в форме тезисов публикуются результаты исследований участников Всероссийской (с международным участием) конференции Российского нейрохимического общества «RUSNEUROCHEM 2022», представленные на конференции в виде устных докладов и стендовых сообщений. В сборнике освещен широкий спектр современных исследований, проводимых ведущими российскими и иностранными учеными-нейрохимиками и специалистами из смежных областей биологии.
Сборник представляет интерес как для специалистов в области изучения биохимии мозга, молекулярных механизмов функционирования нервной системы, патогенеза и терапии нейродегенеративных заболеваний, так и для представителей общего биологического профиля, аспирантов и студентов ВУЗов.
Материалы публикуются в авторской редакции.

This issue presents in the form of abstracts the results of the studies of the participants of the Conference of the Russian Neurochemical Society "RUSNEUROCHEM 2022", given at the Conference in the form of oral reports and poster presentations. The publication comprises a wide range of up-to-date data presented by leading Russian and foreign neurochemists and specialists from related fields of biology.
The publication is of interest both for specialists in the field of brain biochemistry, molecular mechanisms of nervous system functioning, pathogenesis and therapy of neurodegenerative diseases, as well as for researchers of general biological profile, graduate and undergraduate students.

Materials are published in the author's unedited version.

Редакторы сборника: д.б.н. М.Л. Фирсов, д.б.н. Н.Н. Наливаева, к.б.н. О.В. Кручинина
Корректура к.б.н. О.С. Алексеева

Конференция проводится при финансовой поддержке Конгрессно-выставочного бюро Санкт-Петербурга и НЦМУ Павловский центр «Интегративная физиология –медицине, высокотехнологичному здравоохранению и технологиям стрессоустойчивости» по Соглашению № 075-15-2022-296 от 15.04.2022 г.

ISBN 978-5-4386-2177-5

БЕЛОК ЧЕЛОВЕКА SLURP-1 ТОРМОЗИТ МИГРАЦИЮ КЛЕТОК ПЕРВИЧНЫХ ЛИНИЙ МЕЛАНОМЫ ПОСРЕДСТВОМ ВЗАИМОДЕЙСТВИЯ С НИКОТИНОВЫМИ РЕЦЕПТОРАМИ

Кириченко А.В.[1,2], Бычков М.Л.[2], Шлепова О.В.[1,2], Михайлова И.Н.[3], Кирпичников М.П.[2,4], Шулепко М.А.[2], Люкманова Е.Н.[2,4]

1 - Московский физико-технический институт, Москва, Россия
2 - Институт биоорганической химии им. М. М. Шемякина и Ю. А. Овчинникова РАН, Москва, Россия
3 - Национальный медицинский исследовательский центр онкологии им. Н. Н. Блохина, Москва, Россия
4 - Междисциплинарная научно-образовательная школа Московского университета «Молекулярные технологии живых систем и синтетическая биология», Биологический факультет, Московский государственный университет им. М.В. Ломоносова, Москва, Россия

bittert@mail.ru

Активация никотиновых ацетилхолиновых рецепторов (nAChR) способствует пролиферации и миграции раковых клеток и подавляет апоптоз. Секретируемый белок человека SLURP-1, принадлежащий семейству Ly-6/uPAR, ингибирует nAChR типа a7 (a7-nAChR), является ауто/паракринным регулятором гомеостаза эпителиальных клеток и подавляет пролиферацию клеток карциномы. Вместе с этим, экспрессия SLURP-1 снижена в клетках меланомы по сравнению со здоровыми тканями, а также в метастатических клетках меланомы по сравнению с первичной опухолью.

В данном исследовании мы проанализировали экспрессию SLURP-1 и α7-nAChR в образцах опухолевых и нормальных тканей из базы данных TCGA Melanoma и выявили корреляцию между низкой экспрессией α7-nAChR и положительным прогнозом по выживаемости пациентов с меланомой. Мы получили рекомбинантный аналог человеческого SLURP-1 (rSLURP-1) и изучили его антипролиферативную активность на наборе первичных клеточных линий меланомы, включающем одну умеренно дифференцированную клеточную линию (mel P) и две низкодифференцированные клеточные линии (mel Kor, mel H). Мы обнаружили, что rSLURP-1 не оказывает антипролиферативного действия на все исследуемые клеточные линии, однако вызывает арест клеточного цикла клеток mel P в фазе G0/G1. В то же время rSLURP-1 селективно ингибирует миграцию клеток mel P с EC_{50} 67,8 ± 0,4 нМ. Нокдаун экспрессии α7-nAChR в клетках mel P с помощью α7-siRNA полностью отменял влияние rSLURP-1 на миграцию, что указывает на вовлеченность этого рецептора в механизм действия регулятора. Чтобы понять механизм селективности rSLURP-1, был проведен ПЦР-анализ в реальном времени. Все исследуемые линии демонстрировали сходный уровень экспрессии мРНК генов *CHRNA7* и *SLURP1,* однако наблюдалась разница в экспрессии гена *CHRFAM7A,* кодирующего усеченную субъединицу dupα7, которая способна

формировать с полноразмерными субъединицами α7-nAChR нефункциональный гетеромерный рецептор α7-nAChR/dupa7. Таким образом, rSLURP-1 ингибирует миграцию умеренно-дифференцированной меланомы путем взаимодействия с α7-nAChR на поверхности раковых клеток и отсутствие влияния рекомбинантного препарата на остальные исследуемые линии меланомы может быть связано с повышенным содержанием на поверхности их клеток химерных рецепторов α7-nAChR/dupa7, нечувствительных к действию rSLURP-1. Работа выполнена при поддержке Российского научного фонда (проект 17-74-20161).

ВКЛАД TRP КАНАЛОВ В АКТИВНОСТЬ ТРИГЕМИНАЛЬНОГО НЕРВА МЕНИНГЕАЛЬНОЙ ОБОЛОЧКИ КРЫСЫ

Федорина А.И.[1], Ананьев А.С.[1], Телина Э.Н.[2], Гафуров О.Ш.[1]

1 - ФГАОУ ВО «Казанский (Приволжский) федеральный университет», Казань, Россия
2 - ФГБОУ ВО «Казанский государственный медицинский университет», Казань, Россия

alyon.fedorina@mail.ru

Мигрень - изнурительное неврологическое расстройство, пусковым механизмом которого может являться активация волокон тройничного нерва. Повышение температуры, характерное для воспалительного процесса, зачастую сопровождается головными болями, поэтому мы попытались оценить вклад TRP каналов в частоту возникновения потенциалов действия (ПД) в модели мигрени в тройничном нерве менингеальной оболочки крысы.

С помощью внеклеточной регистрации ПД от пучка нервных волокон мы регистрировали частоту возникновения ПД при последовательном повышении и фиксации температуры омывающего раствора на уровне 27^{o}C, 37^{o}C и с аппликацией капсаицина в концентрации 1μM. Использование кластерного анализа позволило оценить вовлеченность отдельных нервных волокон в частоту возникновения ПД.

В результате повышения температуры до 27^{o}C частота ПД достоверно возрастала по сравнению с контролем ($p=0.005$). Дальнейшее повышение температуры до 37^{o}C приводило к достоверному увеличению частоты ПД ($p=0.03$). Однако последующая аппликация капсаицина в концентрации 1μM не приводила к достоверному увеличению частоты возникновения ПД.

Кластерный анализ зарегистрированных ПД в тригеминальном нерве показал, что частота ПД в отдельных нервных волокнах неодинакова. Рассчитав количество волокон, в которых частота ПД увеличилась в 2 раза под воздействием температуры 27^{o}C, 37^{o}C или капсаицина, мы выяснили следующее: 17% волокон не ответили; 10% ответило только на

капсаицин; 10% только на температуру 37°C; 24% только на температуру 27°C; и 39% волокон ответило на 27°C и на 37°C.

Таким образом, можно сделать вывод, что различные волокна, входящие в состав тригеминального нерва, содержат различное сочетание TRP каналов, определяющее их реакцию на различную температуру. Можно предположить, что такая сложная организация терморегуляции в менингеальной оболочке может иметь побочные эффекты, приводящие к возникновению болевой активности и, в том числе, к мигрени.

Работа поддержана грантом президента РФ МК-4585.2022.1.4 и выполнена в рамках Программы стратегического академического лидерства Казанского (Приволжского) федерального университета.

ВЛИЯНИЕ ОКСИДА АЗОТА НА СОСТОЯНИЕ ТУЧНЫХ КЛЕТОК ОБОЛОЧЕК ГОЛОВНОГО МОЗГА КРЫСЫ

Королёва К.С., Нурмиева Д.А., Шайдуллов И.Ф.

Казанский Федеральный Университет, Казань, Россия

k.s.koroleva@yandex.ru

Оксид азота (NO), наряду с монооксидом углерода (CO) и сероводородом (H_2S), представляет собой небольшую газообразную сигнальную молекулу, участвующую в различных биологических процессах. Одна из основных функций NO заключается в регуляции тонуса сосудов, однако известно, что нитроглицерин, донор NO, является триггером мигрени и широко используются для моделирования мигрени как у человека, так и у животных, что предполагает участие компонентов сигнального каскада NO в патогенезе мигрени. В оболочках головного мозга обнаружено плотное скопление тучных клеток вдоль сосудов и нервных окончаний. Тучные клетки содержат в своих везикулах разнообразные про-воспалительные соединения, которые могут влиять на возбудимость нервных окончаний. В тоже время нервные окончания также могут стимулировать тучные клетки, вызывая их дегрануляцию, формируя так называемый нейро-иммунный синапс. Было показано, что тучные клетки играют одну из ведущих ролей в поддержании длительной болевой импульсации во время приступа мигрени, однако неоднозначны данные о влиянии оксида азота, триггера мигрени, на степень дегрануляции тучных клеток. Целью работы является исследование влияние экзогенного NO на состояние тучных клеток оболочек головного мозга крысы. В качестве объекта исследования был использован получереп крысы, в котором были сохранены не тронутыми твердая мозговая оболочка, сосуды, нервные окончания и тучные

клетки. Для исследования влияния NO на дегрануляцию тучных клеток был использован гистологический метод окрашивания оболочек голоного мозга крысы Толуидиновым синим. В качестве донора NO был использован нитропруссид натрия (НПН) в концентрации 200 мкМ. Добавление его в перфузируемый раствор приводило к увеличению частоты потенциалов действия в афферентах тройничного нерва, что свидетельствует об его участие в формировании ноцицептивного сигнала. Однако инкубация получерепа крысы в растворе, содержащем НПН в течение 30 мин не оказывала влияния на морфологию и структуру тучных клеток. Таким образом, «острые» про-ноцицептивные эффекты экзогенного донора NO не связаны с дегрануляцией тучных клеток, но может непосредственно влиять на нервные окончания.

Работа выполнена за счёт средств РНФ № 21-75-00042.

ВОДОРАСТВОРИМЫЙ АНАЛОГ НЕЙРОМОДУЛЯТОРА ЧЕЛОВЕКА LYNX1 ТОРМОЗИТ РАЗВИТИЕ ГЛИОМ IN VITRO И IN VIVO

Бычков М.Л.[1], Шлепова О.В.[1,2], Бабенко В.А.[3], Силачев Д.Н.[3], Павлюков М.С.[1], Шулепко М.А.[1], Яшин К.С.[4], Медяник И.А.[3], Плотников Е.Ю.[3], Кирпичников М.П.[1,5], Шенкарев З.О.[1], Люкманова Е.Н.[1,5]

1 - Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А.Овчинникова Российской академии наук, Москва, Россия
2 - Московский физико-технический институт (Национальный исследовательский университет), Москва, Россия
3 - НИИ физико-химической биологии имени А.Н. Белозерского МГУ имени М.В. Ломоносова, Москва, Россия
4 - Приволжский исследовательский медицинский университет Министерства здравоохранения Российской Федерации, Нижний Новгород, Россия
5 - Междисциплинарная научно-образовательная школа Московского университета «Молекулярные технологии живых систем и синтетическая биология», Биологический факультет, Московский государственный университет им. М.В. Ломоносова, Москва, Россия

maksim.bychkov@gmail.com

Никотиновый рецептор типа α7 (α7-nAChR) вовлечен в развитие многих карцином, тогда как его роль в прогрессировании глиомы остается малоизученной. Lynx1 человека представляет собой GPI-заякоренный модулятор α7-nAChR, экспрессированного на поверхности клеточной мембраны, и его участие в контроле прогрессии рака легкого было описано ранее. В нашей работе мы изучали роль α7-nAChR и Lynx1 в развитии глиом. Для этого мы использовали модельные линии глиом, первичные линии глиом и астроцитов, образцы пациентов с глиобластомой (ГБМ) и модели мышей с имплантированной в головной

мозг глиомой С6. В отличие от нормальных клеток головного мозга и карциномы легкого, Lynx1 в клетках глиомы имел внутриклеточную, а не мембранную локализацию. При этом, в образцах пациентов с ГБМ уровень мРНК Lynx1 был значительно снижен по сравнению с окружающей тканью. Эти данные позволили предположить нарушение модуляции α7-nAChR на поверхности глиомных клеток. Для подтверждения этой гипотезы мы использовали водорастворимый аналог Lynx1 человека (ws-Lynx1) для компенсации отсутствия мембраносвязанного модулятора и восстановления регуляции α7-nAChR в клетках глиобластомы. Обработка клеток глиом препаратом ws-Lynx1 подавляла экспрессию α7-nAChR на клеточной мембране и ингибировала рост и миграцию клеток глиомы, но не первичных астроцитов. Кроме того, при инкубации с ws-Lynx1 в клетках глиом наблюдались остановка клеточного цикла, апоптоз и снижение секреции клетками глиомы фактора воспаления TNF-α. Интраназальное введение ws-Lynx1 мышам с трансплантированной глиомой С6 в течение десяти дней приводило к значительному снижению роста опухоли, что было подтверждено с помощью МРТ. Наши данные указывают на ключевую роль α7-nAChR в прогрессии глиом, и модуляция функции этого рецептора аналогами Lynx1 является многообещающей стратегией терапии глиом.

Работа выполнена при финансовой поддержке РФФИ № 18-29-01050 и стипендии Президента РФ для поддержки молодых учёных СП-1900.2021.4

ДИФФЕРЕНЦИАЛЬНОЕ ВЛИЯНИЕ ПТСР И ДЕПРЕССИИ ОТЦОВ ПЕРЕД ЗАЧАТИЕМ НА АКТИВНОСТЬ ГИПОТАЛАМО-ГИПОФИЗАРНО-АДРЕНОКОРТИКАЛЬНОЙ СИСТЕМЫ ПОТОМКОВ: ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ

Ордян Н.Э., Пивина С.Г., Холова Г.И., Акулова В.К., Ракицкая В.В.

ФГБУН Институт физиологии им. И.П. Павлова РАН, Санкт-Петербург, Россия

neo@infran.ru

В настоящее время растет число доклинических и эпидемиологических наблюдений о трансгенерационном влиянии стресса отца на различные функции потомков без вовлечения прямого взаимодействия между потомками и отцом. Эпидемиологические исследования родителей, страдавших посттравматическим стрессовым расстройством (ПТСР), продемонстрировали усиление ПТСР-подобных симптомов у их потомков, которые сами по себе никакие травматические события не испытывали. В экспериментах, где самцов мышей подвергали хроническому стрессированию в течение всего периода сперматогенеза, установлено снижении стрессорной реактивности гипоталамо-гипофизарно-

адренокортикальной системы (ГГАС) у потомков обоего пола. Однако ПТСР у больных создает сниженный уровень глюкокортикоидных гормонов в крови, а экспериментальные данные о влиянии моделирования ПТСР у отцов на активность ГАС потомков отсутствуют. Возникает вопрос: сама ли по себе стрессорная процедура, предъявленная отцам, влияет на активность ГГАС потомков, или значение имеет тот уровень кортикостерона, который в крови отцов создается в период созревания сперматозоидов? В связи с этим цель исследования состояла в сравнении эффектов ПТСР-подобного состояния (сниженный уровень кортикостерона, парадигма «стресс-рестресс») и депрессивно-подобного состояния (повышенный уровень кортикостерона, парадигма «выученная беспомощность») самцов крыс перед спариванием на активность ГГАС их половозрелых потомков обоего пола. Помимо активности ГГАС у потомков анализировали экспрессию глюкокортикоидых рецепторов (ГР) методом количественной иммуноцитохимии в гиппокампе и медиальной префронтальной коре (мПФК).

Установлено, что у самок – потомков отцов с ПТСР-подобным или депрессивно-подобным состоянием - наблюдается снижение стрессорной реактивности ГГАС и укоренное ее торможение после стрессорной активации в ответ на 30-мин иммобилизацию, что сопровождалось увеличением экспрессии ГР в зубчатой извилине и 2-ом слое мПФК. Схожий профиль активности ГГАС был выявлен и у самцов – потомков отцов с моделированием ПТСР. Однако у самцов – потомков отцов с депрессивно-подобным состоянием – чувствительность ГГАС к сигналам обратной связи снижалась и сопровождалась уменьшением экспрессии ГР в СА1 поле гиппокампа, зубчатой извилине и 2-ом слое мПФК. Сделано заключение, что ПТСР- или депрессивно-подобное состояние отцов в период сперматогенеза оказывает дифференциальное влияние на активность ГГАС и экспрессию ГР в мозге их потомков самцов.

И.П. АШМАРИН И КАФЕДРА ФИЗИОЛОГИИ ЧЕЛОВЕКА И ЖИВОТНЫХ МГУ

Дубынин В.А.

Московский государственный университет им. М.В. Ломоносова, Москва, Россия

dva-msu@yandex.ru

В течение 20 лет (1986-2006) кафедрой физиологии человека и животных биологического факультета МГУ руководил академик РАМН Игорь Петрович Ашмарин. В выступлении рассматривается его выдающаяся роль в качестве ученого, организатора, педагога, создавшего в МГУ школу разностороннего и методического изучения регуляторных

пептидов. Объектами исследования стали десятки природных и искусственно модифицированных пептидных молекул, их воздействие на сердечно-сосудистую систему, пищеварение и обмен веществ, гормональную сферу, нервно-мышечное сопряжение и др. При этом главнейшее внимание уделялось и продолжает уделяться системному рассмотрению выявляемых физиологических, патофизиологических и потенциально лечебных (корректирующих и протекторных) эффектов, механизмам нейроиммуноэндокринного взаимодействия на самых разных уровнях - от молекулярно-генетического до поведенческого.

ИСПОЛЬЗОВАНИЕ ПРОТЕАСОМНОГО ИНГИБИТОРА ЛАКТАЦИСТИНА ДЛЯ МОДЕЛИРОВАНИЯ ДОКЛИНИЧЕСКОЙ И КЛИНИЧЕСКОЙ СТАДИЙ БОЛЕЗНИ ПАРКИНСОНА У КРЫС: РАННИЕ НЕЙРОФИЗИОЛОГИЧЕСКИЕ МАРКЕРЫ

Екимова И.В., Белан Д.В., Гузеев М.А., Пази М.Б., Пастухов Ю.Ф.

Институт эволюционной физиологии и биохимии им. И.М. Сеченова РАН, Санкт-Петербург, Россия

irina-ekimova@mail.ru

Болезнь Паркинсона (БП) — социально-значимое медленно прогрессирующее нейродегенеративное заболевание преимущественно пожилых людей. Клинически значимыми симптомами БП являются двигательные дисфункции, которые могут сопровождаться широким спектром немоторных симптомов. БП остается неизлечимым заболеванием в первую очередь из-за поздней диагностики. Хотя нейропротекторное лечение возможно на доклинической стадии, в настоящее время не существует надлежащего клинического подхода к ранней диагностике БП. Более того, современная терапия не противодействует прогрессированию БП и носит лишь симптоматический характер. Разработка валидных моделей БП на животных, воспроизводящих стадии заболевания, может ускорить решение этих важных вопросов.

Патоморфологическим признаком БП является накопление и агрегация белка α-синуклеина (α-син) в дофаминергических нейронах черной субстанции (ЧС). Убиквитин-протеасомная система (УПС) препятствует патологической агрегации α-син, осуществляя своевременную деградацию аномального α-син. Открытие функциональной недостаточности УПС у пациентов с БП мотивировало разработку нового поколения моделей у грызунов, основанных на введении ингибитора протеасом лактацистина (ЛЦ). Согласно гипотезе двойного удара (Hawkes, Tredici & Braak, 2007), нейротропный патогенный фактор может проникать в мозг через носовой путь и через желудочно-кишечный тракт. С помощью

интраназального введения ЛЦ нам удалось воспроизвести у крыс среднего и пожилого возраста не только клиническую, но и доклиническую стадии БП, отражающие ключевые патофизиологические признаки БП. Модель доклинической стадии БП характеризовалась: допороговым уровнем нейродегенерации и снижения содержания дофамина в нигростриатной системе по сравнению с уровнем клинической стадии; дегенерацией экстранигральных структур; наличием в нейронах агрегатов α-син. Подобно БП, развитие этих патологических процессов сопровождалось микроглиолизом в ЧС и повышением уровня провоспалительных цитокинов ФНО-α и ИЛ-1β в периферической крови, которые могут рассматриваться как возможные потенциальные биомаркеры доклинической стадии. Новая модель БП воспроизводила нарушения сна и эмоционального поведения, которые могут найти применение в клинических исследованиях для ускоренного поиска вероятных немоторных симптомов ранней стадии БП. Однако пока остается неясным насколько уязвима к ЛЦ периферическая нервная система, вовлекаемая в патологию при БП. Несмотря на нерешенные вопросы ЛЦ модель БП может быть хорошей платформой для поиска ранних маркеров и новых нейропротективных соединений. *Работа поддержана госзаданием (№ АААА-А18-118012290427-7).*

ИССЛЕДОВАНИЕ КЛЕТОЧНО-МОЛЕКУЛЯРНЫХ МЕХАНИЗМОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ НЕЙРОДЕГЕНЕРАЦИИ И ПРИМЕНЕНИИ МЕМАНТИНА

Черноморец И.Ю.[1], Першина Е.В.[1], Жуйкова Н.С.[1], Гарджук А.А.[2]

1 - Институт теоретической и экспериментальной биофизики РАН, Пущино, Россия
2 - Московский государственный университет им. М.В. Ломоносова, Москва, Россия

yrgen@mail.ru

В работе моделирование нейродегенерации осуществляли введением половозрелым самцам крыс линии Вистар нейротоксиканта хлорида триметилолова (ТМТ) в дозе 7,5 мг/кг, в/б (n=12). Контрольным животным аналогичным способом вводили физиологический раствор (n=6). Животным группы ТМТ вводили неконкурентный антагонист НМДА рецепторов мемантин гидрохлорид в дозе 10 мг/кг, в/б, через 48 и 72 часа после ТМТ (n=6). Через 21 после инъекции ТМТ оценивали поведение животных, гибель нейронов, локализацию и морфологию глиальных клеток, изменение уровней мРНК генов *EAAT2, Grin2b, IL1b, GFAP, Aif1, TGFb1, TGFbR1, TGFbR2* в гиппокампе и префронтальной коре.

Морфологические исследования выявили снижение плотности нейронов в полях CA4 и CA3 дорсального гиппокампа в группе ТМТ по сравнению с крысами контрольной группы

и животными группы ТМТ+мемантин. В префронтальной коре, преимущественно в IV-V слоях, ТМТ также привел к гибели нейронов, хотя и к менее выраженной, чем в гиппокампе у группы ТМТ+мемантин. Иммунофлуоресцентное окрашивание на микроглию выявило ее активацию в поле CA4 гиппокампа и в областях M2, Cg1 и PrL префронтальной коры как у группы ТМТ, так и у животных группы ТМТ+мемантин.

Поведенческий тест пассивного избегания выявил нарушение долговременной памяти у животных группы ТМТ при сравнении с контролем, в то время как применение мемантина приводило к увеличению времени нахождения животных в светлом отсеке по сравнению с группой ТМТ.

Результаты ОТ-ПЦР в реальном времени показали, что экспрессия генов-маркеров эксайтотоксичности (*EAAT2, Grin2b*) через 3 недели после ТМТ не отличалась от контроля как в гиппокампе, так и префронтальной коре животных групп ТМТ и ТМТ+мемантин. В этот же срок в гиппокампе экспериментальных групп были повышены уровни мРНК для генов-маркеров нейровоспаления (*IL1b, GFAP, Aif1*). При этом в префронтальной коре была повышена экспрессия лишь *GFAP*. Уровень мРНК противовоспалительного цитокина *TGFb1* был повышен в гиппокампе, но не префронтальной коре групп ТМТ и ТМТ+мемантин. Уровень экспрессии одного из рецепторов цитокина – *TGFbR1* повышался лишь в гиппокампе крыс группы ТМТ+мемантин.

Работа поддержана РНФ №21-75-00106.

ИММУНОФЕНОТИПИЧЕСКАЯ КАРТА НЕЙРОГЕНЕЗА КОРЫ ГОЛОВНОГО МОЗГА ЧЕЛОВЕКА

Прощина А.Е., Харламова А.С., Отлыга Д.А., Сонин Г.А., Кривова Ю.С., Юнеман О.А., Цветкова Е.Г., Савельев С.В.

Научно-исследовательский институт морфологии человека им. академика А.П. Авцына, Москва, Россия

proshchina@yandex.ru

Проблема формирования коры большого мозга – одна из центральных тем нейробиологии развития, так как морфологические и функциональные особенности плащевых структур лежат в основе интеллекта и сознания человека. Детальное изучение раннего созревания переднего мозга человека необходимо как для понимания нормального функционирования зрелой структуры, так и для понимания и прогнозирования ряда патологических процессов и состояний. Современные передовые исследования больше сосредоточены на поиске закономерностей функционирования транскриптома

развивающегося мозга. Однако наличие продуктов транскрипции генома не всегда приводит приводит к корректной трансляции, а следовательно, исключительно по анализу транскрипционной активности не всегда возможно судить о функционировании (и даже наличии) белкового продукта в клетке. Исследования протеома развивающегося мозга человека эпизодичны и несистематизированы. Целью проекта, который мы хотим здесь представить, является регион-специфический анализ нейро- и глиогенеза во время нормального внутриутробного развития переднего мозга человека и составление иммунофенотипической карты этих событий на пре-, ранне, средне и позднефетальном этарах развития. Мы планируем сосредоточить свои усилия на изучении клеточной дифференцировки с использованием маркеров, специфичных для нейральной и глиальной линий, а также антител к факторам транскрипции и функциональных маркеров нервной системы. Это позволит получить принципиально новую информацию о протеоме развивающегося мозга и региональной специфике роста и дифференцировки.

Отдельной задачей проекта будет создание открытой цифровой базы, размещенной в сети интернет. В ходе иммуноморфологического исследования фетального аутопсийного материала генерируется большой массив изображений. Эти изображения содержат существенные данные, которые не могут быть включены в формат публикаций и поэтому остаются недоступны исследователям и врачам, работающим как над проблемами нормального нейрогенеза, так и патогенеза врожденных неврологических заболеваний. При этом информация по трансляционному профилю популяций клеток коры головного мозга человека во время внутриутробного развития на современных сетевых ресурсах отсутствует. Мы ожидаем, что подобный ресурс будет востребован в качестве сравнительного материала и руководства для целого ряда разделов практической медицины, образовательных программ и фундаментальных исследований.

Проект (№ 22-15-00172) поддержан РНФ.

КАЛЬЦИЕВЫЙ ИМИДЖИНГ — ТЕХНОЛОГИЯ БУДУЩЕГО ДЛЯ ДИАГНОСТИКИ РАКА ЖЕЛУДКА

Калустова П.В., Варчич А.Э., Романюк Р.А., Сурков М.С., Трепалина В.А., Лысенко Л.В.

Научно-исследовательский технологический Центр нейротехнологий Южного федерального университета, Ростов-на-Дону, Россия

kalustova@sfedu.ru

С развитием медицины появляется потребность в разработке новых методов диагностики онкологии на ранних стадиях. Исследования показали, что повышенная концентрация определённых биомаркеров — летучих органических соединений (ЛОС) в выдыхаемом воздухе может быть верным диагностическим признаком для быстрого неинвазивного анализа проб выдыхаемого воздуха. Для данного исследования был выбран 6-метил-5-гептен-2-он, один из биомаркеров рака желудка (РЖ).

Целью исследований было определение потенциальной возможности нахождения «отпечатка» биомаркера, микроанатомическое представительство которого в карте активности гломерул оптимально совпадало бы с характерными «отпечатками» в картах активности гломерул на образцы воздуха больных онкологическими заболеваниями, на примере рака желудка.

В нашем нейрооптическом исследовании проведен сравнительный анализ ответов гломерул дорсальной поверхности обонятельной луковицы крыс Rattus norvegicus (n=6) на образцы воздуха, выдыхаемого больными онкопатологиями и здоровыми людьми в возрасте 38-50 лет, включая образцы воздуха здоровых испытуемых с добавлением биомаркеров. Сопоставление карт вероятности стимул-специфичной реакции на воздух, выдыхаемый здоровыми испытуемыми и больными раком желудка, продемонстрировало смещение паттерна активности на воздух, выдыхаемый больными раком желудка, при этом микроанатомические регионы больных и здоровых испытуемых значительно перекрываются в диапазоне от 8,2 мм до 10,2 мм по x и от 0,6 мм до 1,0 мм по y относительно брегмы.

Добавление биомаркера рака желудка 6-метил-5-гептен-2-она в образцы воздуха, выдыхаемого здоровыми испытуемыми, частично воспроизводит паттерн, характерный для рака желудка и активирует те же гломерулы, что и воздух, выдыхаемый больными раком желудка. При этом увеличение концентрации 6-метил-5-гептен-2-она в ряде случаев как увеличивало индивидуальную амплитуду ответа гломерул, так и вызывало более слабые ответы гломерул на карте активности, что может быть отражением вариабельности функционального состояния животного.

Исследование выполнено при финансовой поддержке Программы «Стратегическое академическое лидерство» Южного федерального университета («Приоритет 2030»).

Исследование выполнено за счет гранта Российского научного фонда № 22–25-00683, https://rscf.ru/project/22-25-00683/ в Южном федеральном университете.

КОЛИЧЕСТВЕННОЕ ОПРЕДЕЛЕНИЕ НИЗКОМОЛЕКУЛЯРНЫХ МЕТАБОЛИТОВ ПЛАЗМЫ КРОВИ И ТКАНЕЙ ОПУХОЛИ И ПРЕТУМОРАЛЬНОЙ ЗОНЫ ДЛЯ ПРОФИЛИРОВАНИЯ ГЛИОМ

Кочаровская М.В.[1,2], Парамонов А.С.[1], Медяник И.А.[3], Яшин К.С.[3], Шенкарев З.О.[1]

1 - Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А.Овчинникова Российской академии наук, Москва, Россия
2 - Московский физико-технический институт (национальный исследовательский университет), Москва, Россия
3 - Приволжский исследовательский медицинский университет Министерства здравоохранения Российской Федерации, Нижний Новгород, Россия

kocharovskaya.mv@phystech.edu

Глиомы, включая глиобластомы, являются самыми частыми и наиболее агрессивными опухолями головного мозга. Сложность лечения глиом связана с их диффузным ростом, отсутствием четких границ между опухолью и здоровой тканью, а также с большой вероятностью перерождения глиом в более агрессивные глиобластомы. Опухолевые клетки характеризуются значительными перестройками метаболических путей. Исследования методами метаболомики дают информацию о биохимических процессах, протекающих в клетках, поэтому профилирование метаболитов является перспективным подходом для диагностики и прогноза течения различных заболеваний.

Нами была разработана методика анализа профиля низкомолекулярных метаболитов в плазме крови пациентов и в тканях опухоли и претуморальной зоны после резекции опухоли, используя метод ЯМР-спектроскопии. Был проведен подбор условий и созданы протоколы пробоподготовки. Также были оптимизированы условия съемки ЯМР-спектров, отнесения сигналов метаболитов в спектрах и получения их концентраций.

Анализ ЯМР-спектров с использованием информации о химических сдвигах из базы данных HMDB (Human Metabolome Database), а также дальнейшая обработка исходных данных для статистического анализа позволили идентифицировать и определить концентрации для 49 веществ в плазме крови и 43 веществ в тканях опухоли и претуморальной зоны, которые присутствуют в большинстве образцов. Исходные концентрации метаболитов были проанализированы с помощью разработанного для этой цели метода линейных комбинаций. Анализ с помощью этого метода позволил выявить достоверные ($p<0.05$) различия между группами образцов, сформированных по разным

признакам: класс злокачественности глиом, IDH-статус, их комбинация и сравнение с контрольной группой здоровых добровольцев. Полученные данные позволили определить набор метаболитов, которые в наибольшей степени определяют эти различия. Анализ тканей претуморальной зоны у людей разных возрастов позволил выявить метаболиты, которые достоверно различаются у групп пациентов младше и старше 45 лет. С помощью t-критерия Стьюдента было показано различие ($p<0.05$) в концентрациях мио-инозитола, глутамата, о-ацетилкарнитина, глицина, аспартата, аскорбата, НАД+, лейцина, гуанозина, изолейцина и валина.

Таким образом в работе была показана возможность профилирования глиом по профилю метаболитов в тканях опухоли, претуморальной зоне и плазме крови. Работа выполнена при финансовой поддержке РФФИ №18-29-01050.

МИКРОРНК-ОПОСРЕДОВАННАЯ ГИПОТАЛАМИЧЕСКАЯ РЕГУЛЯЦИЯ СТАРЕНИЯ

Маслюков П.М.

Ярославский государственный медицинский университет, Ярославль, Россия

masliukov@rambler.ru

Гипоталамус — филогенетически старый отдел промежуточного мозга, который играет важную роль в поддержании постоянства внутренней среды и обеспечении интеграции функций автономной, эндокринной, соматической систем. Доказано участие гипоталамуса в механизмах старения, при этом важная роль отводится дорсомедиальному (ДМЯ) ядру гипоталамуса.

Целью исследования являлся анализ изменений нейрохимического состава, нейронной активности и уровня микроРНК в ДМЯ гипоталамуса, а также оценке влияния микроРНК на биохимические маркеры старения в плазме крови с использованием иммуногистохимических, электрофизиологических методов, вестерн-блоттинга и ПЦР-РТ.

Результаты показали, что при старении происходят разнонаправленные изменения нейрохимического состава ДМЯ крыс, а также снижение частоты импульсации нейронов ДМЯ. При этом наблюдалось статистически значимое снижение экспрессии let-7a-5p, miR-9a-3p, miR-132-3p и miR-218a-5p преимущественно у самцов. В свою очередь, у старых самцов крыс под влиянием миметиков микроРНК let-7a-5p, miR-9a-3p, miR-132-3p и miR-218a-5p при введении в ДМЯ нормализовалось содержание маркеров возрастных изменений (уровень С-реактивного белка, миоглобина, гормона роста, фактора роста фибробластов

FGF21 в плазме крови, в то время как введение ингибиторов оказывало противоположный эффект. При введении ингибиторов микроРНК в ДМЯ у старых самцов наблюдалась значительная гибель самцов по сравнению с самками, а также в сравнении с более молодыми животными.

Таким образом, ДМЯ гипоталамуса влияет на регуляцию процессов старения за счет снижения выделения специфических микроРНК.

Исследование выполнено за счет гранта Российского научного фонда (проект №19-15-00039).

НЕЙРОХИМИЧЕСКАЯ ШКОЛА САНКТ-ПЕТЕРБУРГСКОГО ГОСУНИВЕРСИТЕТА

Ещенко Н.Д., Галкина О.В.

Санкт-Петербургский государственный университет, Биологический факультет, кафедра биохимии, Санкт-Петербург, Россия

n.ezhenko@spbu.ru

В докладе представлены основные этапа развития работ по изучению биохимии мозга в Санкт-Петербургском государственном университете, начиная с момента организации в 1928 году кафедры биохимии. Первым заведующим кафедры был профессор Е.С. Лондон, который благодаря разработанному им методу синусостомии, первый в мире в экспериментах на интактных животных установил чрезвычайно интенсивное потребление глюкозы головным мозгом. Пионерские исследования профессора Г.Е. Владимирова в условиях высокогорья позволили получить ценные сведения о губительном действии гипоксии на функциональную деятельность ЦНС.

Г.Е. Владимиров совместно с профессором М.И. Прохоровой были инициаторами использования в научной работе сотрудников кафедры различных радиоактивных изотопов (P^{32}, C^{14} и др.), причем радиоактивный углерод был применен для изучения метаболизма в организме животных впервые в нашей стране. В 1963 г. организована проблемная лаборатория биохимии нервной системы, где в течение многих лет проводились углубленные исследования специфических липидов мозга и изучение особенностей углеводного и энергетического метаболизма. Эти работы позволили доказать принципиально важную роль липидов в деятельности мозга, а также продемонстрировали, что гликоген в мозге выполняет не только роль инертного запасного вещества, но и активно участвует в обеспечении функциональной активности ЦНС.

Большое внимание развитию нейрохимии на кафедре уделял профессор И.П.

Ашмарин; особо следует отметить разработанный им цикл лекций, посвященный одному из самых сложных и загадочных разделов биохимии мозга — проблемам памяти; эти материалы отражены в пособии «Загадки и откровения биохимии памяти» (1975). По инициативе проф. И.П. Ашмарина были подготовлены и выпущены несколько учебников и учебных пособий по нейрохимии.

В докладе приводятся сведения о том, как в настоящее время на кафедре биохимии проводится подготовка молодых специалистов - бакалавров, магистров, аспирантов, интересующихся проблемами нейрохимии (авторские лекционные курсы, практикумы, примеры тематики выпускных квалификационных работ).

НОВЫЕ ПОДХОДЫ ДЛЯ ВОССТАНОВЛЕНИЯ ФУНКЦИЙ ГИПОТАЛАМО-ГИПОФИЗАРНО-ГОНАДНОЙ ОСИ ПРИ МЕТАБОЛИЧЕСКИХ РАССТРОЙСТВАХ

Шпаков А.О.

Институт эволюционной физиологии и биохимии им. И.М. Сеченова, Российская академия наук, Санкт-Петербург, Россия

alex_shpakov@list.ru

Гипоталамо-гипофизарно-гонадная (ГГГ) ось играет ключевую роль в контроле репродуктивных функций у мужчин и женщин. В качестве основных функциональных звеньев она включает (1) гипоталамические нейроны, секретирующие гонадолиберин, релизинг-фактор лютеинизирующего (ЛГ) и фолликулостимулирующего гормонов (ФСГ), (2) гонадотропоциты аденогипофиза, продуцирующие ЛГ и ФСГ, а также (3) гонады (семенники, яичники), в которых осуществляется синтез половых стероидных гормонов. Наряду с гормонами, продуцируемыми звеньями ГГГ оси, имеется широкий спектр других эндогенных регуляторов, влияющих на секрецию гонадолиберина, гонадотропинов, андрогенов и эстрогенов, среди которых адипокины (лептин, адипонектин, резистин, висфатин и др.), инкретины (глюкагоноподобный пептид-1, грелин), пептиды инсулинового семейства (инсулин, инсулиноподобный фактор роста-1, инсулиноподобный пептид-3), гонадотропин-ингибирующий гормон, активины, ингибины, фоллистатин. Эти регуляторы оказывают на ГГГ ось разнонаправленные влияния, а нарушения в их сигнальных путях приводят к широкому спектру репродуктивных дисфункций. Так лептин положительно влияет на все звенья ГГГ оси. Однако в условиях характерной для ожирения и диабета 2-го типа гиперлептинемии, следствием которой является лептиновая резистентность, функционирование ГГГ оси нарушается. Важное значение для восстановления

репродуктивной системы имеют фармакологические препараты, стимулирующие различные звенья ГГГ оси. Значительный интерес здесь представляют низкомолекулярные аллостерические регуляторы рецептора ЛГ, в том числе производные тиено[2,3-d]-тиенопиримидина, и их комбинации с хорионическим гонадотропином человека (ХГЧ) и метформином. Нами показано, что тиено[2,3-d]-тиенопиримидиновые производные с активностью агонистов рецептора ЛГ стимулируют стероидогенез и нормализуют сперматогенез у самцов крыс в норме, в условиях диабетической патологии и при старении, а также усиливают стероидогенные эффекты ХГЧ.

Работа поддержана Российским Научным Фондом (проект № 19-75-20122).

ОПТОГЕНЕТИЧЕСКАЯ СТИМУЛЯЦИЯ ПАРВАЛЬБУМИНОВЫХ ИНТЕРНЕЙРОНОВ ЭНТОРИНАЛЬНОЙ КОРЫ И ГИППОКАМПА МОДИФИЦИРУЕТ ЭПИЛЕПТИФОРМНУЮ АКТИВНОСТЬ В СРЕЗАХ МОЗГА МЫШИ

Трофимова А.М., Постникова Т.Ю., Проскурина Е.Ю., Зайцев А.В.

Институт эволюционной физиологии и биохимии им. И.М. Сеченова РАН, Санкт-Петербург, Россия

alina.trofimova1132@mail.ru

Введение. Низкочастотная электрическая стимуляция мозга используется для подавления судорожной активности у людей с эпилепсией. Однако терапевтический результат может быть улучшен при определении типов клеток, участвующих в подавлении судорог. В данной работе, используя оптогенетическую стимуляцию, мы исследовали роль активации парвальбуминовых (PV) интернейронов на эпилептиформную активность в гиппокампе и энторинальной коре мозга мыши.

Методы. Работа была выполнена на 4-месячных мышах B6.129P2-*Pvalb*$^{tm1(cre)Arbr}$/J (JacksonLab), экспрессирующих Cre-рекомбиназу в PV-интернейронах. Мышам по стереотаксическим координатам (AP: -4 мм, ML: 3,5 мм, DV: -3,5 мм) вводили аденоассоциированный вектор (AAV9-EF1a-DIO-hChR2(H134R)-mCherry), несущий ген каналородопсина 2 типа (ChR2), в поле CA1 гиппокампа на границе с латеральной энторинальной корой (ЛЭК). Эксперименты проводили через 4–5 недель на переживающих срезах головного мозга. Активацию интернейронов, экспрессирующих ChR2, проводили светом с длинной волны 470 нм с использованием лазерного диод-волоконного источника света. Эпилептиформную активность вызывали в срезе аппликацией проэпилептического раствора с 4-аминопиридином (100 мкМ). Биофизические свойства PV-интернейронов,

экспрессирующих ChR2, регистрировали методом патч-кламп в режиме «целая клетка». Регистрацию полевых потенциалов производили в поле CA1 гиппокампа и/или энторинальной коре в зависимости от локализации экспрессии ChR2.

Результаты. Фотостимуляция даже небольшой интенсивности достаточна для генерации потенциалов действия в ChR2-экспрессируемых PV-интернейронах поля CA1 гиппокампа. Минимальная длительность фотостимуляции, необходимая для стабильной генерации одного потенциала действия, равна 0,5 мс. Определены оптимальные параметры фотостимуляции для воздействия на эпилептиформную активность в разных областях среза мозга. В поле CA1 гиппокампа оптимальная частота, задающая интериктальную активность, равна 1 Гц; минимальная длительность светового стимула – 10 мс. В энторинальной коре оптимальная частота фотостимуляции PV-интернейронов, задающая ритмическую интериктальную активность, равнялась 0,33 Гц; при этом высокие частоты стимуляции (от 1 Гц и выше) не влияли на протекание эпилептической активности в срезе. После окончания оптогенетической стимуляции PV-интернейронов в гиппокампе нередко наблюдалась иктальная активность.

Заключение. Таким образом, PV-интернейроны оказывают влияние на ритм интериктальной активности, однако их активация не приводит к существенному ослаблению или усилению эпилептиформной активности в срезах мозга.

Работа поддержана грантом РФФИ 19-315-60016

ОБНОВЛЕННАЯ МЕТОДОЛОГИЯ РАЗРАБОТКИ ДОКЛИНИЧЕСКОЙ ДИАГНОСТИКИ БОЛЕЗНИ ПАРКИНСОНА

Блохин В.Е., Угрюмов М.В.

Институт биологии развития им. Н.К. Кольцова РАН, Москва, Россия

victor.blokhin@hotmail.com

Введение: Ранняя диагностика болезни Паркинсона (БП) является важной частью изучения БП. Поскольку это длительное заболевание для его диагностики необходима широкая панель маркёров, однако важно, чтобы эти маркёры сохранялись как на клинической так и на доклинической стадии развития БП.

Цель: Целью данной работы являлся поиск биомаркёров в плазме у пациентов в группе риска и их валидация на моделях доклинической и клинической стадии БП.

Материалы и методы: Использовались самцы линии C57Bl/6 в возрасте 8 недель. Для моделирования доклинической стадии БП мышам единожды вводили 18 мг/кг МФТП, а

для моделирования клинической стадии делали 3 инъекции по 10 мк/кг с интервалом в 2 часа. Материал собирали через 2 недели. Для моделирования В группу испытуемых включались люди прошедшие неврологическое обследование. Из крови испытуемых и моделей БП выделяли лимфоциты и плазму. Лимфоциты использовали для ПЦР анализа, а плазму для измерения моноаминов и метаболитов, аминокислот, уратов и индекса оксидативного стресса (ИОС).

Результаты: При анализе плазмы у людей в группе риска было обнаружено снижения уровня L-ДОФА и ДОФУК, тенденция к повышению уровня 5-гидрокситриптофана. Также было выявлено повышение ИОС и снижение концентрации уратов в плазме. Не наблюдались изменения концентраций таурина, глицина, глютаминовой и аспарагиновой кислот. В лимфоцитах уровни экспрессии рецепторов Д3 и Д4 снижались, однако достоверных значений не было обнаружено из-за небольшой выборки испытуемых.

Анализ плазмы моделей доклинической и ранней клинической стадии БП показал, что на доклинической стадии наблюдается снижение уровня L- ДОФА и ДОФУК, а на ранней клинической стадии добавляются снижения уровня дофамина, 3-О-метилдофы и 3-метокситирамина. Отмечается увеличения уровня серотонина, а 5-гидрокситриптофан не изменяется. ИОС на доклинической стадии имел тенденцию к снижению, а на ранней клинической стадии увеличивался относительно контроля. В отличии от группы риска наблюдалось увеличение концентрации глицина в плазме на ранней клинической модели и тенденция к увеличению на доклинической модели БП. Концентрация уратов в плазме не изменялась. Было отмечено снижение экспрессии в лимфоцитом рецепторов Д3 и Д4 на ранней клинической стадии, на доклинической стадии значения экспрессии были на уровне контролей.

Вывод: L-ДОФА и ДОФУК являются потенциальными маркёрами для диагностики БП, так как в плазме как на моделях БП так и в группе риска происходит снижение их уровня. Также потенциальными маркёрами следует считать изменения экспрессии уровня Д3 и Д4 рецепторов, а также изменение ИОС. Последний может быть использован как дополнительный маркёр.

ОСЬ ГИПОТАЛАМУС-ЖКТ У БОЛЬНЫХ ОЖИРЕНИЕМ – ГДЕ НАЧАЛО ПРОБЛЕМЫ?

Бабенко А.Ю.

НЦМУ ЦПМ НМИЦ им. В.А. Алмазова, Санкт-Петербург, Россия

alina_babenko@mail.ru

Ожирение характеризуется нарушением регуляции аппетита и пищевого поведения (ПП), наличие которых ухудшает возможность снижения веса. В наше исследование были включены 53 пациента с ожирением, средний возраст 38,7±10,8 лет, средняя масса тела (сМТ) 102,8±16,4 кг, индекс массы тела (ИМТ)=36,8±4,6 кг/м2), всеми пациентами были заполнены опросники, в соответствии с дизайном исследования: Голландский опросник пищевого поведения (Dutch Eating Behavior Questionnaire (DEBQ)), визуально-аналоговая шкала (ВАШ) состоящая из 4 вопросов (Насколько голодным вы себя чувствуете? Насколько сытым вы себя чувствуете? Насколько сильно вы хотите есть? Сколько пищи вы могли бы сейчас съесть?). В связи с тем, что по данным DEBQ у большинства пациентов были выявлены несколько типов нарушений ПП, все пациенты были разделены в группы с доминантным типом ПП. Средний % пациентов с доминирующим эмоциогенным типом нарушения пищевого поведения (ЭмПП) составил 72,2%, с экстернальным типом (ЭксПП) - 11,1%, ограничительного ОПП +16,7%, то есть с наибольшей частотой встречался эмоциогенный тип ПП, ассоциированный со стрессовыми воздействиями и наиболее сложный в плане коррекции. Анализ гормональных изменений продемонстрировал повышение уровня глюкозозависимого инсулинотропного пептида (ГИП) натощак и отсутствие его дальнейшего прироста в постпищевом статусе (после стандартного углеводистого завтрака), что указывает на резистентность к данному инкретину. Учитывая ключевую роль этого инкретина в депонировании жира в подкожном депо, резистентность к нему может определять перераспределение депонирования жира в висцеральное депо. Одновременно отмечен более низкий, чем у людей с нормальной массой тела, уровень глюкагоноподобного пептида 1 (ГПП-1), инкретина, вовлеченного в регуляцию аппетита. Дефицит ГПП-1 является одним из важных факторов, ассоциированных с развитием нарушения регуляции чувства голода/насыщения, пищевого поведения. Его уровень идентифицирован нами как предиктор ответа на терапию арГПП-1, препаратами, нормализующими чувство голода/насыщения благодаря стимуляции рецепторов ГПП1 в гипоталамусе. Современные исследования однозначно указывают на то, что питание, богатое жирами и углеводами с высоким гликемическим индексом изменяет состав микробиома ЖКТ, что в свою очередь приводит к нарушениям продукции инкретинов, аналогичным тем,

что были обнаружены в нашем исследовании. Таким образом, ось нездоровое питание-изменение состава микробиома-нарушение инкретинового баланса – нарушение пищевого поведения является одним из ключевых механизмов, лежащих в основе развития ожирения.

Исследование выполняется при поддержке гранта МинОбра РФ (Соглашение No. 075-15-2020-901).

ОЦЕНКА ТРЕВОЖНОСТИ МЫШЕЙ С ХРОНИЧЕСКОЙ ПОСТ-ВОСПАЛИТЕЛЬНОЙ МОДЕЛЬЮ СИНДРОМА РАЗДРАЖЕННОГО КИШЕЧНИКА

Муллакаева А.И., Салихзянова А.Ф., Яруллина Д.Р., Яковлева О.В.

Казанский федеральный университет, Казань, Россия

adelya.may.of@gmail.com

Микробиота кишечника представляет собой сложное видовое сообщество микроорганизмов, населяющих экосистему желудочно-кишечного тракта. Клинические и доклинические исследования показывают, что изменения кишечной микробиоты влияют не только на различные кишечные расстройства, но и на заболевания ЦНС, в том числе связанные с неврологическими заболеваниями человека и животных. В нашей работе мы анализировали тревожно-фобическое состояние мышей с пост-воспалительной моделью синдрома раздраженного кишечника (СРК).

Для моделирования пост-воспалительного СРК самцов белых мышей опытной группы (n = 10) ежедневно в течение 11 дней подвергали неонатальной сенсибилизации путем интраректального введения разведенного 1 % раствора уксусной кислоты (0.3 мл в возрасте 10-14 дней и 0.5 мл в возрасте 15-21 дней). Мыши контрольной группы получали эквивалентный объем физиологического раствора (n = 10). Через 2 недели оценивали тревожно-фобическое состояние животных с помощью тестов «Интегральный показатель тревожности» и «черно-белая камера». На 45 день эксперимента исследовали изменение состава кишечной микрофлоры методами классической микробиологии. Моделирование СРК не приводило к достоверному снижению молочнокислых бактерий, в том числе лактобацилл, в фекалиях по сравнению с контрольными животными. Но у мышей опытной группы в кишечнике практически полностью исчезали лактозоположительные энтеробактерии и оставались только лактозоотрицательные Salmonellla spp. и Shigella spp.

Тест «черно-белая камера» основан на врожденной неприязни грызунов к ярко освещенным областям, поэтому применяется для оценки уровня тревожности. Время нахождения в световой камере в контрольной группе составило 75.3 ± 11.7 с. В опытной

группе этот показатель снизился до 48.1 ± 19.5 с ($p < 0.05$), что указывает на повышенный уровень тревожности. Мыши опытной группы также демонстрировали высокий уровень тревожности в тесте «Интегральный показатель тревожности» (1.10 ± 0.23 балл, $p < 0.05$), относительно контрольной группы (0.20 ± 0.08 балл).

Полученные нами результаты свидетельствуют о неблагоприятных изменениях в кишечной микробиоте сопровождающихся увеличением тревожности при пост-воспалительном хроническом СРК.

Работа поддержана грантом РНФ № 22 -25-20045.

ПОДАВЛЕНИЕ РОСТА КАРЦИНОМ СИНТЕТИЧЕСКИМ ФРАГМЕНТОМ SLURP-1 ЧЕЛОВЕКА, ДЕЙСТВУЮЩИМ НА НИКОТИНОВЫЕ АЦЕТИЛХОЛИНОВЫЕ РЕЦЕПТОРЫ α7 ТИПА

Шулепко М.А.[1], Бычков М.Л.[1], Шлепова О.В.[1,2], Кульбацкий Д.С.[1], Чулина И.А.[3], Азев В.Н.[3], Кошелев С.Г.[1], Кирпичников М.П.[1,4], Шенкарев З.О.[1,2], Люкманова Е.Н.[1,2,4]

1 - Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А.Овчинникова, Москва, Россия
2 - Московский физико-технический институт (национальный исследовательский университет), Долгопрудный, Россия
3 - Группа химии пептидов, филиал Института биоорганической химии им. академиков М.М. Шемякина и Ю.А. Овчинникова, Пущино
4 - Междисциплинарная научно-образовательная школа Московского университета «Молекулярные технологии живых систем и синтетическая биология», Биологический факультет, Московский государственный университет имени М.В. Ломоносова, Москва, Россия

mikhailshulepko@gmail.com

Одним из перспективных направлений для разработки новых противоопухолевых препаратов является регуляция работы никотиновых ацетилхолиновых рецепторов (nAChR), в особенности α7 типа (α7-nAChR). Эти рецепторы вовлечены в процессы регуляции пролиферации, миграции и апоптоза раковых клеток, а экспрессия гена *CHRNA7*, кодирующего α7 субъединицу nAChR, повышена в клетках карцином. SLURP-1 – это секретируемый белок, экспрессирующийся в эпителии человека и принадлежащий семейству трехпетельных белков Ly6/uPAR. SLURP-1 является селективным негативным аллостерическим модулятором α7-nAChR и подавляет рост клеток карцином кожи, кишечника и легкого, в том числе предотвращая пролиферацию клеток, индуцированную никотином.

Механизм действия рекомбинантного аналога SLURP-1 человека был исследован в клетках аденокарциномы легкого A549. SLURP-1 ингибировал пролиферацию и миграцию

клеток A549. SLURP-1 снижал фосфорилирование PTEN и киназы mTOR, что указывает на подавление сигнального пути PI3K/AKT/mTOR. Также наблюдались снижение фосфорилирования тромбоцитарного рецептора фактора роста типа β (PDGFRβ) и остановка клеточного цикла в фазах S и G2/M без индукции апоптоза. Методом аффинной экстракции показано, что SLURP-1 образует комплекс не только с α7-nAChR, но также с PDGFRα и рецептором эпидермального фактора роста (EGFR), которые, как известно, участвуют в регуляции роста и миграции раковых клеток и способны образовывать гетеродимеры. Нокдаун генов, кодирующих α7-nAChR, PDGFRα и EGFR, подтвердил участие этих рецепторов в антимиграционном эффекте SLURP-1. Таким образом, мишенью действия SLURP-1 в мембране эпителиальных клеток могут быть комплексы α7-nAChR с PDGFRα и EGFR. Используя химерные белки с пересаженными петлями SLURP-1, мы показали, что петля I является основным активным центром, ответственным за взаимодействие SLURP-1 с α7-nAChR и его антипролиферативное действие. Синтетический пептид, имитирующий петлю I, стабилизированный дисульфидной связью, ингибировал вызванный ацетилхолином ток через канал α7-nAChR, а также пролиферацию и миграцию клеток A549. Этот синтетический пептид, получивший название «Онкотаг» представляет собой перспективный прототип нового противоопухолевого препарата со свойствами, близкими к нативному белку SLURP-1.

Работа выполнена при финансовой поддержке РВК (Центр НТИ ИБХ РАН № 2/1251/2018) и РНФ (№ 17-74-20161).

ПОИСК, РАЗРАБОТКА И ВАЛИДАЦИЯ ПОТЕНЦИАЛЬНЫХ ФАРМАКОЛОГИЧЕСКИХ СРЕДСТВ НА ОСНОВЕ ПРОИЗВОДНЫХ ПИПЕРАЗИНОВ ДЛЯ ОГРАНИЧЕНИЯ СИНАПТИЧЕСКОЙ УТРАТЫ В НЕЙРОНАХ ГИППОКАМПА

Попугаева Е.А.[1], Унанян Л.С.[2], Зернов Н.И.[1], Макичан А.Т.[2], Камарян В.С.[2], Мелентьева Д.М.[1]

1 - Санкт-Петербургский политехнический университет Петра Великого, Санкт-Петербург, Россия
2 - Российско-Армянский университет, Ереван, Армения

lena.popugaeva@gmail.com

Прогрессивная синаптическая утрата в нейронах гиппокампа на ранних стадиях отличает болезнь Альцгеймера (БА) от других нейродегенеративных заболеваний. Ранее нами было показано, что ограничение синаптической утраты с помощью воздействия на нейроны гиппокампа соединением 51164, производным пиперазина, способно замедлить

темпы развития БА в in vitro и in vivo моделях. 51164 является положительным регулятором активности каналов TRPC6, входящих в состав депо-управляемых кальциевых каналов. 51164 помогает восстановить синаптическую пластичность в резах мозга мышей линии 5xFAD (Popugaeva et al 2019, Mol Pharm.). Однако при проведении доклинических исследований обнаружилось, что соединение 51164 не стабильно в плазме крови и плохо проницает через ГЭБ.

В рамках настоящей работы были применены методы молекулярного докинга и конформационного анализа 51164 и гиперфорина с каналом TRPC6 для выявления ключевых сайтов и типов взаимодействия соединений с активным центром канала TRPC6 (Hunanyan et al 2021, IJMS). На основании полученных in silico данных был определен фармакофор, N-, N-замещенный пиперазин, который был использован для поиска химических соединений структурно схожих с фармакофором и соответствующих критериям «подобия лекарства».

С помощью in vitro исследований было определено соединение лидер, демонстрирующее специфичность в отношении каналов TRPC6. Проведены пилотные доклинические исследования, определена in vitro стабильность соединения в плазме крови мыши и человека. В первичной культуре гиппокампа установлен нейропротекторный эффект соединения лидера в концентрации 100нМ. В срезах мозга мышей 5xFAD исследовано влияние соединения на синаптическую пластичность.

При успешном прохождении доклинических исследований in vivo (стабильность в плазме крови, проницаемость через ГЭБ, острая токсичность) соединение лидер может быть рассмотрено в качестве потенциального фармакологического средства для лечения болезни Альцгеймера.

Работа выполнена при поддержке Российского научного фонда (номер проекта 20-75-10026).

РЕГУЛЯЦИЯ СЕКРЕЦИИ МЕДИАТОРА В НОВООБРАЗОВАННЫХ МОТОРНЫХ СИНАПСАХ МЫШИ С УЧАСТИЕМ ПРОБДНФ И ПРОДОМЕНА БДНФ

Богачева П.О., Правдивцева Е.С., Гайдуков А.Е., Балезина О.П.

ФГБОУ ВО Московский государственный университет имени М.В. Ломоносова, Москва, Россия

untergang@inbox.ru

Нейротрофин мозга (БДНФ) хорошо известен как регулятор развития и дифференцировки нейронов и модулятор синаптической передачи в ЦНС. Кроме того, БДНФ

рассматривается и как миокин, высвобождаемый из мышцы и оказывающий ретроградные потенцирующие влияния на моторные синапсы. Наряду со зрелым БДНФ, одновременно могут оказывать действие и продукты его созревания – проБДНФ и продомена БДНФ. Возможность миогенного высвобождения проБДНФ описана в периферических моторных синапсах мыши в период неонатального развития скелетных мышечных волокон, а также и в зрелых скелетных мышцах в ответ на их денервацию. Однако роль проБДНФ и образующегося в ходе его процессинга продомена БДНФ и направленность их ретроградных влияний на новообразованные в ходе реиннервации мышцы моторные синапсы, остаются не изученными.

В связи с этим, целью работы было выявить влияния и механизмы действия проБДНФ и продомена БДНФ на секрецию ацетилхолина в регенерирующих моторных синапсах.

Исследования проводили на синапсах m. EDL на 11 сутки после передавливания малоберцового нерва. При помощи стандартной микроэлектродной техники отведения биопотенциалов регистрировали и анализировали возникающие под влиянием проБДНФ и продомена БДНФ изменения параметров спонтанных миниатюрных потенциалов концевой пластинки (МПКП) и вызванных потенциалов концевой пластинки (ПКП), генерируемых в режиме коротких ритмических залпов.

В новообразованных синапсах проБДНФ вызывал увеличение мембранного потенциала мышечных волокон и снижение частоты МПКП на 35%, которые предотвращалось тертиапином-Q (блокатором калиевых каналов GIRK), но не ингибитором сигналинга рецепторов p75 TAT-Pep5. При этом проБДНФ не вызвал сдвигов амплитуды и квантового состава ПКП.

Продомен БДНФ оказывал собственные эффекты: увеличивал амплитуду МПКП, что предотвращалось везамиколом (ингибитором везикулярного транспортера ацетилхолина) и уменьшал квантовый состав ПКП.

Таким образом впервые было показано, что проБДНФ и продукт его созревания продомен БДНФ при действии на новообразованные моторные синапсы способны к самостоятельным влияниям на параметры спонтанной и вызванной секреции медиатора. Реализация таких влияний обеспечивается за счет активации каналов GIRK и усиления накачки ацетилхолина в везикулы, соответственно.

Работа поддержана грантом РНФ 22-25-00111.

РОЛЬ К(АТФ)-КАНАЛОВ В ЭФФЕКТАХ H_2S И NO В ТРОЙНИЧНОМ НЕРВЕ КРЫСЫ

Буглинина А.Д., Верхотурова Т.М., Гафуров О.Ш., Королёва К.С., Ситдикова Г.Ф.

ФГАОУ ВО «Казанский (Приволжский) федеральный университет», Казань, Россия

verrrwolf@gmail.com

Мигрень – нервно-сосудистое заболевание, характеризующееся пульсирующей головной болью, зачастую сопровождающуюся фоно- и фотофобией. Данное заболевание представляет собой одну из актуальнейших проблем медицины, универсального подхода к лечению которого пока не удалось найти. Ряд клинических исследований позволил установить ключевую роль тройничного нерва и менингеальных сосудов в патогенезе мигрени. В волокнах тройничного нерва и тригеминоцервикальном комплексе обнаружена экспрессия Kir6.1, Kir6.2, SUR1 и SUR2 субъединиц $К^+$(АТФ)-каналов. В контексте тригемино-васкулярной теории происхождения боли при мигрени, предполагается, что активация $К^+$(АТФ)-каналов приводит к гиперполяризации мембраны, с последующей активацией циклических нуклеотид-управляемых (HCN) каналов. Открытие этих каналов способствует деполяризации мембраны и увеличению частоты потенциалов действия в нейронах. Однако в менингеальных нервных окончаниях вклад $К^+$(АТФ)-каналов в ноцицептивную активность не показан.

В связи с этим целью данной работы является оценка роли $К^+$(АТФ)-каналов в формировании ноцицептивного сигнала в возбудимости менингеальных афферентов, а также анализ их участия в про-ноцицептивных эффектах газообразных посредников, таких как донор H_2S (гидросульфит натрия) и донор NO (нитропруссид натрия) в тройничной системе крысы.

Эксперименты проводились на самцах (4–8 недель) крыс линии Wistar с использованием в качестве объекта исследования препарата изолированного черепа крысы. Периферический отросток тройничного нерва помещался в регистрирующий электрод для последующей передачи и оцифровки полученных данных о спайковой активности. Было установлено, что неспецифический блокатор глибенкламид (1мМ, n=5) вызывает достоверное увеличение частоты потенциалов действия, тем самым опосредуя ноцицептивный эффект. Тогда как на фоне глибенкламида был зафиксирован про-ноцицептивный эффект донора NO (n=4), увеличения частоты потенциалов действия в ответ на аппликацию донора H_2S при тех же условиях не наблюдалось. Это указывает на то, что одной из мишеней действия сероводорода могут выступать $К^+$(АТФ)-каналы.

Таким образом, $К^+$(АТФ)-каналы участвуют в модуляции возбудимости нейронов, а

также являются мишенью действия для донора H_2S.

Работа выполнена при финансовой поддержке РНФ №20-15-00100.

СРАВНИТЕЛЬНЫЙ АНАЛИЗ СТРУКТУРНО-ДИНАМИЧЕСКИХ ОСОБЕННОСТЕЙ ТРЕХПЕТЕЛЬНЫХ БЕЛКОВ - МОДУЛЯТОРОВ НИКОТИНОВЫХ АЦЕТИЛХОЛИНОВЫХ РЕЦЕПТОРОВ

Парамонов А.С.[1], Заиграев М.М.[1,2], Кочаровская М.В.[1,2], Кульбацкий Д.С.[1], Шулепко М.А.[1], Чугунов А.О.[1], Люкманова З.О.[1,3], Шенкарев З.О.[1]

1 - Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А.Овчинникова Российской академии наук, Москва, Россия
2 - Московский физико-технический институт (Национальный исследовательский университет), Москва, Россия
3 - Междисциплинарная научно-образовательная школа Московского университета «Молекулярные технологии живых систем и синтетическая биология», Биологический факультет, Московский государственный университет им. М.В. Ломоносова, Москва, Россия

apar@nmr.ru

Никотиновые ацетилхолиновые рецепторы (nAChRs) представляют собой лиганд-зависимые ионные каналы. Известно, что дисфункция nAChR способствует ряду заболеваний центральной и периферической нервной системы, таких как болезнь Альцгеймера, паркинсонизм, миастения, эпилепсия, депрессия, никотиновая и алкогольная зависимости, а также, возможно, некоторым раковым заболеваниям и заболеваниям иммунной и эндокринной системы. Белки семейства Ly6/uPAR, имеющие характерную “трехпетельную” архитектуру, представляют собой эндогенные модуляторы nAChR и играют важные и разнообразные роли во многих процессах в нервной системе. Так, для них показана способность к регуляции когнитивных процессов, антипролиферативная активность и участие в ряде регуляторных путей, таких как каскад Wnt/β-катенин.

Для выявления структурных и динамических особенностей, отвечающих за взаимодействия белков из семейства Ly-6/uPAR с nAChR, был проведён комплексный анализ структуры и динамики широкого ряда «трехпетельных» белков, действующих на nAChR, как водорастворимых (SLURP-1, SLURP-2), так и изолированных трехпетельных LU-доменов GPI-заякоренных белков (Lypd6, Lypd6b, Lynx1, Lynx2), а также нейротоксинов из яда кобры («слабый» токсин WTX и нейротоксин NT-II). Были рассмотрены структурные особенности строения LU-доменов, выявлены различные типы топологии β-структуры, свойства поверхности и распределения зарядов. По данным ЯМР охарактеризована динамика и конформационная пластичность, которая может быть важной характеристикой,

обеспечивающей адаптацию структур для связывания с различными рецепторами в различных конформационных состояниях и позволяющая контролировать свободную энергию образования комплекса лиганд-рецептор.

Одними из важных особенностей GPI-заякоренных белков являются свойства линкерной области между LU-доменом и GPI-якорем, которые могут влиять на фармакологию трехпетельных белков. В частности, длина и подвижность линкера может определять сайт связывания на рецепторе и способ и аффинность взаимодействия. Было проведено молекулярное моделирование GPI-заякоренных белков Lynx1, Lynx2, Lypd6 и Lypd6b с использованием полноатомной модели нейрональной мембраны.

Полученные данные выявили сложные взаимосвязи между структурой, динамикой и функцией белков Ly6/uPAR, действующих на nAChR. Знание структурно-динамического ландшафта эндогенных трехпетельных модуляторов nAChR будет востребовано в том числе для рационального дизайна лекарств например, с помощью вычислительных методов.

Работа выполнена при поддержке РНФ (грант №19-74-20176).

ТАРГЕТНАЯ ТЕРАПИЯ БОЛЕЗНИ ПАРКИНСОНА

Пчелина С.Н.[1,2], Усенко Т.С.[1,2], Емельянов А.К.[1,2], Рычков Г.Н.[1], Захарова Е.Ю.[3]

1 - НИЦ «Курчатовский институт» - ПИЯФ, Гатчина, Россия
2 - Первый Санкт-Петербургский государственный медицинский университет им. И.П. Павлова, Санкт-Петербург, Россия
3 - Медико-генетический научный центр имени академика Н.П. Бочкова, Москва, Россия

sopchelina@hotmail.com

В последние годы наблюдается прорыв в разработке нейропротекторных препаратов направленного действия (таргетных) для лечения распространенного нейродегенеративного заболевания, болезни Паркинсона (БП). Несколько лекарственных форм находятся в настоящее время на второй стадии клинических испытаний. Разработка данного класса препаратов стала возможна в результате описания молекулярной этиологии развития наследственных форм БП. Наиболее обсуждаемые мишени – лизосомный фермент глюкоцереброзидаза, GBA, мутантные формы которой, ассоциированные со снижением активности фермента, приводят к развитию наследственного заболевания из класса лизосомных болезней накопления, болезни Гоше, и повышают риск развития БП в 8-10 раз, а также обогащенная лейциновыми повторами киназа 2, LRRK2, мутантные формы которой, приводящие к повышению киназной активности, вызывают развитие аутосомно-доминантной формы БП. Целью нашего многолетнего исследования является разработка

таргетных лекарственных препаратов для лечения GBA- и LRRK2-БП.

Методы. С использованием построенной атомарной модели GBA нами проведен молекулярный докинг описанных ранее соединений, связывающихся с GBA и приводящих к повышению активности данного фермента, фармакологических шаперонов (ФШ) GBA, предложены химические модификации соединений, проведен их синтез. Влияние новых ФШ GBA, а также известного ингибитора LRRK2, MLi-2, на активность GBA было протестировано на первичной культуре макрофагов от пациентов с GBA-БП и LRRK2 -БП. Дифференцировку моноцитов в макрофаги проводили с использованием колониестимулирующего фактора макрофагов (М-КСФ) (Sigma-Aldrich, USA) в конечной концентрации 10 нг/мл. Ферментативную активность лизосомных гидролаз оценивали методом тандемного масс-спектрометрического анализа. Эффективность действия MLi-2 на активность LRRK2 оценивали по соотношению нефосфориллированного к фосфориллированному белку rab10 методом вестерн блоттинга с использованием антител MJF-R23, MJF-R21 (Abcam, USA).

Результаты. При воздействии предложенных соединений на первичную культуру макрофагов от пациентов с GBA-БП мы показали статистически значимое повышение ферментативной активности GBA как при воздействии предложенными нами ФШ GBA, так и ингибитором LRRK2. Однако, в то время как ФШ GBA оказывали селективное действие, культивирование макрофагов с MLi-2 приводило к повышению активности ряда лизосомных гидролаз.

Выводы. Нами предложены новые ФШ GBA, показавшие большую эффективность в восстановлении активности GBA по сравнению с исходными соединениями. Впервые на культуре первичных макрофагов показано повышение активности GBA при действии ингибитора LRRK2, однако, в отличие от ФШ GBA действие MLi-2 приводило также к повышению активности и других лизосомных гидролаз.

Благодарности: Исследование выполнено при поддержке грантов РНФ№22-25-00501, РНФ№ 22-25-00721.

УВЕЛИЧЕНИЕ ПРОНИЦАЕМОСТИ ГЕМАТОЭНЦЕФАЛИЧЕСКОГО БАРЬЕРА КРЫС С ПРЕНАТАЛЬНОЙ ГИПЕРГОМОЦИСТЕИНЕМИЕЙ

Деттерер А.С., Яковлев А.В.

Казанский федеральный университет, Казань, Россия

anna.detterer@gmail.com

Гематоэнцефалический барьер (ГЭБ) – структура, обеспечивающая избирательную проницаемость между компонентами крови и паренхимой головного мозга. Гомоцистеин – серосодержащая аминокислота, участвующая в цикле метионина. Повышенное содержание в крови гомоцистеина (гипергомоцистеинемия, ГГЦ) вызывает нарушение ГЭБ, через усиление производства активных форм кислорода и активацию матриксных металлопротеиназ, которые играют важную роль в разрушении белкового матрикса и приводит повышенной проницаемости сосудов. Целью данного исследования стало изучение проницаемости ГЭБ крыс в условиях хронического действия гомоцистеина и его производных.

В экспериментах использовались следующие группы животных: контрольная группа, животные с пренатальной ГГЦ и с введение гомоцистеин-тиалоктона, животные с пренатальной ГГЦ с интраперитонеальным введением N-ацетилцистеин (NAC, 40 мг/кг) и NaHS (3 мг/кг). Для создания хронической модели пренатальной ГГЦ использовалась пищевая метиониновая нагрузка (7.7 г/кг корма) в течение всей беременности самок крыс. Эксперименты проводились на потомстве в возрасте 30-40 дней после рождения. Для оценки проницаемости ГЭБ определили экстравазацию альбумина в ткани головного мозга с использованием красителя Evans Blue (ЕВ, 2мл/кг). Прохождение красителя Evans Blue через ГЭБ оценивали спустя 60 мин после его внутривенного введения. Определение содержания красителя в гомогенате мозжечка животного осуществляли спектрофотометрически (620 нм) с использованием планшетного ИФА ридера Multiscan FS (Thermo scientific, США) по калибровочным кривым. Для анализа действия производных гомоцистеина – гомоцистеин-тиолактон (10 мг/кг) водился подкожно за 60 мин до введения красителя.

Анализ экспериментальных данных показал, что в контрольных условиях после внутривенного введения ЕВ не отмечалось выхода красителя за пределы церебральных сосудов. Концентрация ЕВ в гомогенате клеток мозжечка контрольной группы животных составляла 0.1 ± 0.04 мкг/мг ткани мозжечка ($N = 11$). Предварительное введение гомоцистеин-тиолактона вызывало увеличение проницаемости ГЭБ, концентрация ЕВ составляла 0.4 ± 0.2 мкг/мг ткани ($N = 9$; $p < 0.05$). В условиях пренатальной ГГЦ и в тканях мозга потомства наблюдалось нарушение проницаемости ГЭБ и содержание ЕВ в тканях мозжечка составляла 0.6 ± 0.09 мкг/мг ($N = 8$; $p < 0.05$). Введение животным с пренатальной

ГГЦ как NAC, так NaHS приводило к снижению проницаемости ГЭБ и концентрация ЕВ в гомогенате клеток мозжечка составляла 0.09±0.03 мкг/мг ($N = 5$; $p < 0.05$) и 0.02 ± 0.004 мкг/мг ($N = 5$; $p < 0.05$) соответственно.

Таким образом, полученные данные свидетельствуют о негативном влиянии высокого уровня гомоцистеина и его производных в крови на проницаемость гематоэнцефалического барьера у крыс с пренатальной гипергомоцистеинемией. Возможно, токсическое действие гомоцистеина связано с нарушением структуры эндотелия сосудов мозга, дисфункцией астроцитов и нейронов. Введение NAC или донора сероводорода – NaHS предотвращало негативное воздействие высоких доз гомоцистеина.

Работа была выполнена при поддержке гранта РНФ 20-15-00100.

ХОЛИНЕРГИЧЕСКАЯ РЕГУЛЯЦИЯ РЕЭПИТЕЛИЗАЦИИ РАН С ПОМОЩЬЮ РЕКОМБИНАНТНОГО АНАЛОГА БЕЛКА ЧЕЛОВЕКА SLURP-2 И ЕГО СИНТЕТИЧЕСКИХ АНАЛОГОВ

Шлепова О.В.[1,2], Бычков М.Л.[2], Шулепко М.А.[2], Чулина И.А.[3], Парамонов А.С.[2], Азев В.Н.[3], Кирпичников М.П.[2,4], Шенкарев З.О.[2], Люкманова Е.Н.[2,4]

1 - Московский физико-технический институт (национальный исследовательский университет), Долгопрудный, Россия
2 - Институт биоорганической химии им. академиков М.М. Шемякина и Ю.А. Овчинникова, Москва, Россия
3 - Группа химии пептидов, филиал Института биоорганической химии им. академиков М.М. Шемякина и Ю.А. Овчинникова, Пущино, Россия
4 - Междисциплинарная научно-образовательная школа Московского университета «Молекулярные технологии живых систем и синтетическая биология», Биологический факультет, Московский государственный университет имени М.В. Ломоносова, Москва, Россия

shlepova@phystech.edu

Миграция кератиноцитов контролируется различными факторами роста и сигнальными путями, в том числе с участием никотиновых и мускариновых рецепторов ацетилхолина (nAChR и mAChR, соответственно). Известно, что активация mAChR приводит к усилению роста клеток кожи, а активация nAChR ослабляет миграцию кератиноцитов, нарушая врожденный кожный иммунитет. Таким образом, модуляция nAChR и mAChR в клетках эпителия представляет перспективную стратегию для усиления реэпителизации ран. Известно, что рекомбинантный аналог белка человека SLURP-2 является ингибитором α4β2- и α3β2-nAChR и аллостерическим модулятором M1 и M3 mAChR человека. Взаимодействуя с α3β2-nAChR и M3-mAChR, rSLURP-2 усиливает пролиферацию кератиноцитов человека. Эти данные свидетельствуют о том, что SLURP-2 является важным участником эпителизации

ран и рекомбинантный аналог белка человека SLURP-2, или его миметик, может использоваться для ускорения ранозаживления.

В данной работе мы исследовали влияние rSLURP-2 и его мутантных форм (rSLURP-2[R20A], rSLURP-2[D38A], rSLURP-2[D52A] и rSLURP-2[Y61A]) на миграцию кератиноцитов. Оказалось, что и rSLURP-2, и его мутантные варианты ускоряют миграцию кератиноцитов. При этом мутант rSLURP-2[R20A], с мутацией в «голове» трехпетельной молекулы SLURP-2, демонстрировал большую активность, чем сам rSLURP-2. Количество клеток мигрировавших через базальную мембрану после 24-часовой инкубации с 100 нМ SLURP-2 составило 135,6 ± 9,9 % относительно количества мигрировавших клеток в контроле, и 114,2 ± 5,1% после обработки rSLURP-2[R20A]. Полученные данные указывают на то, «голова» молекулы SLURP-2 является активным сайтом белка. Для подтверждения этого предположения, с помощью твердофазного синтеза были получены небольшие пептидные аналоги, содержащие возможные активные центры белка SLURP-2: участки петель I, II, III и участок из «головы» белка SLURP-2. Мы изучили влияние пептидов на миграцию кератиноцитов и получили, что участок из «головы» белка SLURP-2 является наиболее активным. Кроме того, все пептиды усиливали и пролиферацию кератиноцитов. Полученные результаты свидетельствуют о возможности использования пептидных аналогов rSLURP-2 для ускорения эпителизации ран.

Работа выполнена при финансовой поддержке РВК, Центр НТИ ИБХ РАН № 2/1251/2018.

ХРОНИЧЕСКОЕ НЕДОСЫПАНИЕ И ЕГО ПОСЛЕДСТВИЯ ДЛЯ НЕРВНОЙ, ЭНДОКРИННОЙ И ДРУГИХ СИСТЕМ ОРГАНИЗМА

Екимова И.В.

Институт эволюционной физиологии и биохимии им. И.М. Сеченова Российской академии наук, Санкт-Петербург, Россия

irina-ekimova@mail.ru

После десятилетий научных исследований, направленных на раскрытие биологии сна, в настоящее время практически невозможно отрицать важность сна для жизни животных и человека. Когда мы спим, в нашем теле происходит множество целительных процессов: очистка мозга от метаболитов и амилоидных белков, восполнение запасов АТФ, глюкозы, мобилизация иммунной системы, усиление нейрогенеза. Наутро мы ощущаем восстановление физической работоспособности и улучшение мыслительных способностей.

За последние 100 лет среднесуточное количество сна у людей сократилось с 8 ч до 5 ч и менее. Причины этого очень разнообразны – от экстремальных рабочих графиков и стресса, до добровольного ограничения сна без видимых социальных причин из-за Интернета и телевидения.

Исследования с участием добровольцев демонстрируют, что недосыпание приводит к нарушению сна, внимания, рабочей памяти, развитию депрессии, что указывает на функциональные нарушения в головном мозге, которые в значительной степени не изучены. Проведенные нами исследования на модели хронического недосыпания у грызунов показали, что патоморфологической основой этих нарушений могут являться структурно-функциональные перестройки в дофаминергической мезокортикальной и нигростриатной системах и норадренергической системе голубого пятна вследствие развития нейродегенеративного процесса по PERK/CHOP-зависимому апоптозному пути. Есть убедительные экспериментальные доказательства того, что при хроническом недосыпании необратимой деструкции моноаминергических нейронов может способствовать нарушение энергетического и окислительно-восстановительного гомеостаза вследствие дисфункции митохондрий и нарушение глимфатического клиренса головного мозга. Недостаточный ежедневный сон можно рассматривать как новый фактор риска развития нейродегенеративных заболеваний. Растет количество эпидемиологических данных о связи между уменьшением продолжительности сна и нарушением гормонально-метаболического статуса, который рассматривается как фактор увеличения заболеваемости ожирением, сахарным диабетом 2 типа, гипертонии и эректильной дисфункции. Однако остается целый ряд нерешенных вопросов, позволяющих понять, какие конкретно эндокринные нарушения и механизмы их развития лежат в основе этих заболеваний.

Поддержано грантом на создание и развитие НЦМУ «Павловский центр. Интегративная физиология – медицине, высокотехнологичному здравоохранению и технологиям стрессоустойчивости» (№ 075-15-2020-916 от 13.11.2020).

5-HT3 RECEPTOR-DEPENDENT SUPRASPINAL NEURONAL ALTERATIONS CONTRIBUTING TO INTESTINAL HYPERALGESIA IN COLITIS

Lyubashina O.A.[1,2], Sivachenko I.B.[1], Busygina I.I.[1]

1 - Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia
2 - Valdman Institute of Pharmacology, Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russia

lyubashinaoa@infran.ru

There is clinical and experimental evidence that gut inflammation alters visceral nociceptive processing in the spinal cord and brain, promoting the development of intestinal hyperalgesia–the leading cause of chronic abdominal pain. The serotonin 5-HT3 receptors are involved in visceral nociception at both the peripheral and central levels. Selective 5-HT3 receptor blockers are shown to reduce spinal and brain structures' responses to painful distension of noninflamed bowel. In colitis, the anti-inflammatory action of 5-HT3 antagonists in the periphery has been revealed, but their effects on the pathology-associated central alterations responsible for intestinal hyperalgesia remain unexplored. The objectives of this study were to determine the colitis-induced changes in neuronal properties of the visceral pain-related supraspinal sites and to estimate these alterations under 5-HT3 receptor blockade. In urethane-anaesthetized healthy control and colitis adult male Wistar rats, the extracellular microelectrode recording and electrical microstimulation were used for assessing noxious colorectal distension (CRD)-evoked neuronal responses within studied brain regions and their descending modulation by higher-level structures prior to and after intravenous or intracerebroventricular injection of 5-HT3 antagonist granisetron. In the presence of colitis, an enhancement of CRD-induced neuronal firing was observed in the caudal ventrolateral medulla (CVLM), nucleus of the solitary tract (NTS), and midbrain central gray (MCG). Colitis was also associated with decreased inhibitory effects of the MCG, central amygdala, and infralimbic cortex electrostimulations on the medullary CRD-responsive cells. In these conditions, intravenous granisetron (1-2 mg/kg) dose-dependently suppressed augmented nociceptive activity of the CVLM, NTS and MCG neurons. In turn, intracerebroventricular granisetron administration (20 μg/10 μl) in colitis caused an increase in excitatory amygdalofugal and corticofugal influences on the medullary visceral nociceptive processing. The revealed 5-HT3 receptor-dependent sensitization of the brainstem viscerosensory pathways and impaired 5-HT3-related balance between descending pain inhibition and facilitation can be important factors contributing to intestinal hyperalgesia in colitis.

ANALYSIS OF TYPE 1 MELANOCORTIN RECEPTOR EXPRESSION IN DIFFERENT REGIONS OF MOUSE BRAIN

Romanova I.V., Mikhailova E.V., Mikhrina A.L., Shpakov A.O.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St.Petersburg, Russia

irinaromanova@mail.ru

There are many data on the localization of the melanocortin type 1 receptor (MC1R) at the periphery, while information on the presence of MC1R in different areas of the brain is very scarce. The availability of such information is necessary for understanding the role of brain MC1R in the functioning of neurons and in the central regulation of physiological functions. The aim of this work was to study the expression and distribution of MC1R in different areas of the brain of female C57Bl/6J mice. Using real-time PCR, we have shown the expression of the *Mc1r* gene in the hypothalamus, midbrain, hippocampus, medulla oblongata and cortex. The localization of MC1R in neurons of the hypothalamic arcuate, paraventricular and supraoptic nuclei, in the nucleus of the solitary tract (NTS) and the hippocampus, as well as in the cerebral cortex was demonstrated using an immunohistochemical approach. Using double immunolabeling in the arcuate nucleus, NTS, hippocampus (the CA3 and CA1 regions) and the cerebral cortex, the localization of MC1R on the bodies and outgrowths of pro-opiomelanocortin (POMC - a precursor of melanocortin peptides) immunopositive neurons was shown. Co-localization with POMC may indicate the possibility that MC1R, like MC3R, functions as an autoreceptor, as well as its involvement in both the control of food intake and the neuroendocrine regulation. In the paraventricular and supraoptic nuclei, MC1R localization was demonstrated on the bodies of vasopressin- and oxytocin-immunopositive neurons, which indicates the relationship between MC1R signaling in the hypothalamus and the production of vasopressin and oxytocin. The data obtained indicate the multiplicity of functions of MC1R in the nervous system, including the possibility of MC1R-mediated regulation of the melanocortin, vasopressin, and oxytocin systems in the hypothalamus and other brain areas.

Supported by the Ministry of Science and Higher Education of the Russian Federation grant (№ 075-15-2020-916).

ACTION OF PERMANENT HYPERDOPAMINERGIA ON RATS' BEHAVIOUR: OPERANT CONDITIONING AND PROGRESSIVE RATIO SCHEDULES

Sukhanov I., Savchenko A.

Pavlov medical university, St. Petersburg, Russia

ilia.sukhanov@gmail.com

Although dopamine neurons are comparatively few in number in the human brain, dopamine is critically involved in different functions of the central nervous system related to the control of voluntary movement, affect, reward, sleep, and cognition. The dopaminergic modulation of frontostriatal circuit provides the ability to adapt learning processes on an organism's needs, motivation and reward history. Rats lacking dopamine transporter (DAT-KO) are a promising model for studying these behavioural processes.

DAT-KO rats as well as their heterozygotes and wild-types littermates were tested in a set of experiments evaluating conditional associative learning and goal-directed behaviour. All experiments were performed in standard operant boxes for rats. Pavlovian conditioning seems to be unaffected by the depletion of DAT in rats. However, DAT-KO rats were completely unable to acquire a new operant skill in Pavlovian/Instrumental autoshaping task. The findings in might be explained by both: impaired incentive salience assignment and disturbances of instrumental behaviour acquisition in the KO animals. To discriminate about them, we additionally evaluated the ability of CS to get reinforcing properties by itself (i.e., to become a secondary/conditioned reinforcement and get incentive value) in rats. This process seems to be dramatically disrupted in the DAT-KO rats.

Progressive ratio 3 schedule of food reinforcement, which means the ratio of reinforcement increased by 3 after each earned reinforcement, was used to estimate motivations in DAT-KO rats. We analyzed the dynamics of the local response rate during the session because of its apparent advantages over the classical "breakpoint" approach. The progressive increase of ratio was accompanied by the decrease of the local response rate in the control animals. On the contrary, the local response rate dynamics was dramatically changed in the DAT-KO rats: the response rate was gradually increasing as the required number of responses to obtain a reward was growing.

ACUTE AND LONG-TERM CHANGES IN GENE EXPRESSION IN THE RAT HIPPOCAMPUS IN RESPONSE TO CENTRAL LIPOPOLYSACCHARIDE (LPS) EXPOSURE

Shishkina G.T., Lanshakov D.A., Kalinina T.S., Bannova A.V., Sukhareva E.V., Babenko V.N., Dygalo N.N.

Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia

gtshi@bionet.nsc.ru

Pro-inflammatory stimuli such as lipopolysaccharide (LPS) trigger changes in brain gene expression, which, especially associated with neuroinflammation, can lead to the development of neurological diseases. But over how long, LPS-induced gene alterations present after endotoxin administration, remains yet unknown. In our study, gene expression pattern was investigated in the rat hippocampus 24 hours and 80 days after the central LPS infusion. At 24 h after LPS, 341 differentially expressed genes (DEGs) were detected. The acute alterations of gene expression were related mostly to immune/inflammatory activation and defense responses. During the next period between 24 h and 80 days, LPS-induced changes in expression of 314 genes were disappeared, but remained for 27 genes. At 80 days after LPS, the total number of DEGs was 88, among which 27 DEGs were therefore the same as that at 24 h. Twenty four genes altered their expression in similar direction at both post LPS time points. In contrast, 3 genes (Tfap2b, Shox2 and Syt2) downregulated at 24 hours were significantly upregulated at 80 days. In addition to commonly changed 27 genes, new 61 DEGs were observed at 80 days. This group along with genes associated with immune/inflammatory processes, also included genes related to regulation of neurotransmitter system activities, alterations in which were believed to be involved in development of psychological disturbances. In our study, a significant increase in expression of Slc17a6 that mediates the uptake of glutamate into synaptic vesicles at presynaptic nerve terminals was detected. In contrast, expression of Slc18a1 involved in the transport of biogenic monoamines, such as serotonin, from the cytoplasm into the secretory vesicles was significantly decreased. Delayed changes in expression of both these genes after LPS may be processing through different pathways including modulation of Lmx1a expression.

Supported by grant from the Russian Science Foundation (N 20-64-47013)

ACUTE CORTICOSTERONE ELEVATION IN RATS DEPENDS ON TIME OF THE DAY WHEN LATERAL FLUID PERCUSSION BRAIN INJURY HAS BEEN APPLIED

Bashkatova D.A.[1], Komoltsev I.G.[1,2], Frankevich S.O.[1,2], Shirobokova N.I.[1], Volkova A.A.[1], Shalneva D.V.[1], Kostryukov P.A.[1], Kostyunina O.V.[1], Chizhova O.A.[1], Salyp O.Yu.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

bashkatik2307@mail.ru

Introduction: Lateral fluid-percussion injury (LFPI), a model of traumatic brain injury (TBI) in rats, is accompanied by immediate seizures and corticosterone (CS) elevation in blood and CS accumulation in the hippocampus during the acute posttraumatic period. The aim of this study was to make out whether immediate seizure semiology and CS elevation depends on the time of the day when LFPI is applied.

Materials and methods: The study was performed on 85 male adult Wistar rats. Animals were divided into 3 groups: TBI group (n=36), sham operation group (n=40) and intact control group (n=9). TBI was modeled using LFPI from 9:00 to 22:00. Records of immediate seizures were analyzed. Animals were sacrificed on days 1, 3, 7 and 14 by quick decapitation for biochemical analysis from 10:00 to 12:00. Serum obtained from decapitation blood samples was used for the measurement of CS level with ELISA.

Results: We observed significant correlation between the time of TBI modeling and further CS levels ($r=-0.56$, $p=0.0004$): the earlier time corresponded to higher CS levels in the acute period. Application of k-means clustering method identified 2 distinct clusters based on aforementioned parameters: cluster 1 with "morning TBI" and high decapitation CS level and cluster 2 with "evening TBI" and low decapitation CS level. Clonic seizures were more common and extended in the "morning TBI" cluster.

Conclusion: Time of the day when TBI is modeled considerably affects CS elevation in acute TBI period: earlier LFPI results in higher levels of CS in blood and affects the occurrence of tonic seizures and their duration. Thus, scheduling LFPI at different time of the day represents a tool to model high and low acute corticosterone elevation, which is important to study the involvement of excessive corticosterone in TBI-induced distant hippocampal damage.

Disclosures. Supported by RSF, grant № 22-25-00713.

ADRENERGIC REGULATION OF SODIUM EXCRETION BY THE RAT KIDNEYS

Kutina A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

kutina_anna@mail.ru

In mammals adrenergic innervation influences renal blood vessels and tubules and affects glomerular filtration rate, renin release, and sodium reabsorption. At the same time, the physiological data on the role of the nervous regulation of sodium reabsorption are very contradictory. The aim of this study was to investigate the contribution of α-adrenoreceptors to the regulation of excess sodium excretion and to search for a locus in the nephron responsible for adrenergic effects. The experiments were performed on Wistar rats received oral NaCl load (0.9% NaCl, 50 ml/kg). In these conditions the effects of the inhibitors of α1/2 (phentolamine), α1 (doxazosin) and α2-adrenoreceptors (rauwolscine) were evaluated. After NaCl loading sodium excretion increased significantly from 0.08±0.01 to 1.14±0.19 mmol/h/kg. Phentolamine and doxazosin slow down the excretion of excess sodium and chloride ions by four times, blockade of α2-adrenoreceptors had a lesser effect. Diuretics (furosemide, hypothiazide, amiloride or acetazolamide) were used to search for a locus in the nephron where a regulatory change in sodium reabsorption occurs during blockade of α-adrenergic receptors. Theoretically, during the action of one or another diuretic, a regulatory increase or decrease in sodium transport in the same part of the nephron is impossible. In doxazosin-treated rats furosemide had full effect, whereas natriuretic action of acetazolamide, hypothiazide and amiloride reduced twice. Thus, it is likely that it is in the thick ascending part of Henle loop that the adrenergic regulation of sodium reabsorption occurs. Doxazosin eliminates the inhibitory effect of sympathetic nerves on the Na/K/2Cl-cotransporter activity, and thereby increases sodium retention and reduces the effectiveness of most diuretics. The data obtained indicate the importance of α1-adrenoreceptor-mediated sympathetic regulation of urinary ion excretion.

The study was conducted as part of the state task of the IEPhB RAS.

ALTERATIONS IN BEHAVIOUR AND MICROGLIA MORPHOLOGY IN ADULT RATS AFTER NEONATAL LIPOPOLYSACCHARIDE EXPOSURE

Buianova A.A., Manolova A.O., Nedogreeva O.A., Kvichanskii A.A., Lazareva N.A., Stepanichev M.Yu., Gulyaeva N.V.

Institute of Higher Nervous Activity and Neurophisiology RAS, Moscow, Russia

anastasiiabuianova97@gmail.com

Inflammatory challenge at the early childhood is considered to cause increased vulnerability to depressive-like behaviour in adulthood. Neonatal lipopolysaccharide exposure (NLE) at the first postnatal week may lead not only to transient elevation of glucocorticoid level but also to long term alteration in the glial cells reaction to stress. In the work presented here, we have tested this hypothesis. Wistar rat male pups were injected with E.coli lipopolysaccharide (s.c., 50 mkg/kg) at the 3 and 5 postnatal days (PD), control group obtained equivalent volume of vehicle. At the age of 90PD part of the rats were subjected to behavioural testing including Porsolt test. 30 minutes after the second day of the Porsolt test rats were anesthetized by chloralhydrate and subjected to cardioperfusion. Then the brains were removed and post fixed in formalin fixative. 50 mkm thick coronal sections were immunohistochemically stained using antibodies to markers of microglia (Iba-1, Wako) and astroglia (GFAP, Sigma). Analysis of glial cells state was conducted at the CA1, CA3 subfields and the hilus of the dentate gyrus in the dorsal and ventral parts of the hippocampus. Cell count for both types of glial cells and analysis of fractal dimension and lacunarity for microglial cells were performed. In the Porsolt test the rats subjected to early life proinflammatory stress indeed show a decrease in the time of struggling (control: 163±45 s, stressed: 121±38 s, adjusted p-value=0.048). The astrocytes and microglial cell counts were not influenced by neither NLE nor adult stress or their interaction. NLE and adult stress were not lead to changes in microglia morphology, but their interaction has statistically significant effect (for fractal dimension: $F(1,110) = 4.447$, $p = 0.037$); for lacunarity: $F(1,110) = 7.127$, $p = 0.009$). This fact suggests that NLE modulates the microglial response to stress in adulthood.

ALTERATIONS IN GLUTAMATE METABOTROPIC RECEPTOR GENES EXPRESSION IN THE RAT BRAIN IN THE TEMPORAL LOBE EPILEPSY MODEL

Kovalenko A.A., Zakharova M.V., Schwarz A.P., Zubareva O.E., Dyomina A.V., Zaitsev A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

kovalenko_0911@mail.ru

About 30% of patients with epilepsy suffer from pharmacoresistant forms of the disease. A promising approach is preventing epilepsy development in susceptible groups. Metabotropic glutamate receptors (mGluRs) are involved in epileptogenesis and are therefore suitable targets for new drug development. However, alterations in mGluR gene expression after seizures and the role of these changes in epileptogenesis are still poorly understood. The purpose of this study is to clarify this question. We analyzed RNA expression of mGluRs from groups I (*Grm1*, *Grm5*), II (*Grm2*, *Grm3*), and III (*Grm4*, *Grm7*, *Grm8*) in the dorsal and ventral areas of the hippocampus and temporal cortex 3, 7 (latent phase of the model), and 60 (chronic phase of the model) days after lithium-pilocarpine-induced seizures using real-time RT-qPCR.

Changes in mRNA production of mGluRs differed in the latent and chronic phases of the model. During the latent phase, the most significant changes were found in the gene expression of mGluRs from groups I and III, suggesting their possible involvement in epileptogenesis. In particular, we observed increased expression of the mGluR5 gene in both hippocampus regions but not in the temporal cortex. The gene expression of mGluRs from group III was significantly reduced in all studied regions during the latent phase. Most of the changes in expression detected in the latent phase are not found in the chronic phase or, on the contrary, have changed their direction and become compensatory. However, mGluR8 mRNA production remains reduced in the hippocampus in the chronic phase. The study deepens our understanding of the mechanisms of epileptogenesis and suggests that agents acting on group III mGluRs are the most promising targets for preventing epilepsy development.

Supported by the IEPhB Research Program 075-00408-21-00.

ALTERATIONS IN MICROGLIA AND CX3CR1 EXPRESSION IN RAT HIPPOCAMPUS IN DELAYED TIME PERIODS AFTER NEONATAL LIPOPOLYSACCHARIDE EXPOSURE

Manolova A.O., Buianova A.A., Nedogreeva O.A., Kvichanskii A.A., Lazareva N.A., Stepanichev M.Yu., Gulyaeva N.V.

Institute of Higher Nervous Activity and Neurophisiology RAS, Moscow, Russia

anna.manolova@ihna.ru

Inflammatory challenge at the early childhood is considered to cause increased vulnerability to depressive-like behaviour in adulthood. Neonatal lipopolysaccharide exposure (NLE) at the first postnatal week may lead to long term moderate activation of microglial cells in the hippocampus. In the work presented we tried to reveal whether fraktalkine and its receptor (CX3CR1) take part in such prolong microglial activation. Wistar rat male pups were injected with E.coli lipopolysaccharide (s.c., 50 mkg/kg) at the 3 and 5 postnatal days (PD), control group pups were injected with equivalent volume of vehicle. At the age of the 30PD and 90PD part of the rats were subjected to behavioural testing including Porsolt test. 30 minutes after the second day of the Porsolt test rats were anesthetized by chloralhydrate and subjected to cardioperfusion. Then the brains were removed and divided into hemispheres to make it possible to perform immunohistochemical analysis of hippocampal microglia state and quantitative RT-PCR of the fractalkine and CX3CR1 expression in the same brain.

Porsolt test reveals decrease in the time of struggling swimming in the adult (control: 163±45 s, stressed: 121±38 s, adj. p-value=0.048) but not in the adolescence rats. NLE alone led to moderate activation of microglia only at the age of PD30 ($F(1,9)=8.02$, $p=0.020$). These changes were corroborated with an increase in CX3CR1 expression ($p=0.004$). At the age of PD90 the microglial activation was emerged only in response to combination of NLE and behavioural stress (e.g. Porsolt test) ($F(1,110) = 4.447$, $p = 0.037$). And there was no pronounced alteration in CX3CR1 expression. So long term changes in the microglia activity provoked by the NLE are not always corroborated with the changes in fraktalkine and CX3CR1 expression.

ANTIBODIES AND NEUROCHEMICAL CHANGES IN AUTOIMMUNE ENCEPHALITIS: GOALS FOR THE FUTURE

Fominykh V.[1,2,3], Volik A.[1,4], Yakovlev A.[1], Brylev L.[1,2], Gulyaeva N.[1]

1 - Institut of HigherNervous Acticity and Neurophysiology of RAS, Moscow, Russia
2 - Bujanov Moscow City Clinical Hospital, Moscow, Russia
3 - Kazaryan Center of Epileptology and Neurology, Moscow, Russia
4 - Lomonosov Moscow State University, Moscow, Russia

hydrohinon@mail.ru

Autoimmune encephalitis (AE) is an important consideration in patients with suggested CNS inflammatory disorders and an interesting point for neurochemical studies due to «pure autoimmunity» in some antibodies. These antibodies can be a diagnostic markers or pathological agents in different AE.

Unfortunately current AE diagnostic criteria are very wide. Only 50-60 % of all AE were antibody-positive so far AE diagnosis often based only on clinical findings.

The aim of this study was identification of additional markers which can help differentiate AE from other disorders and can be include to diagnostic panel.

24 AE patients (8 men and 16 women) were studied compare to demyelinating disorders (n=61) and control group(n=25). CSF markers of neurodegeneraton (phosphorylated neurofilament heavy chain, pNfh) and neuroinflammation (interleukin-6 - IL-6, soluble receptor of IL-6, erythropoietin, neopterin, anti ribosomal proteins antibodies) were measured.

In AE group we recognized 2 NMDA, 1 CASPR, 2 LGI1, 1 GABAb, 2 GAD, 1 anti-hu encephalitis. All patients with oncology have Ogbs compare to 13 % positivity in other patients (P=0,0004). Biochemical markers in patients with and without epileptic seizures, with and without known antibodies, with and without Ogbs did not differ from each other. After comparison between groups we revealed higher protein, pNfh and neopterin levels in AE group compare to control but compare to demyelinating group only neopterin level was higher. We found higher CSF IL-6, pNfh, protein and cytosis in patients who died. After univariante logistic regression test significance was found only for protein level.

We characterized clinical and biochemical data in AE group. Ogbs positivity was found in patients with paraneoplastic AE. We revealed neopterin as possible biomarker for AE. Higher pNfh, IL-6, protein levels and cytosis should be revised as bad prognostic markers for AE. For future studies biochemical markers in antibody-defined AE should be investigated.

ANTINOCICEPTIVE EFFECT OF A COMBINATION OF PHYTOCANNABINOIDS AND SNAKE VENOMS

Voskanyan A.V., Parseghyan L.M., Darbinyan A.A., Mogrovyan A.V.

L.A. Orbeli Institute of Physiology of the National Academy of Sciences of Armenia, Yerevan, Armenia

arminvosking@gmail.com

Background: We aimed to study the analgesic and anti-inflammatory effect of oregano essential oil with a high content of beta-caryophyllene and its epoxide, snake venoms, and their combined effect. The analgesic effect of cobra venom has long been known, but a similar effect of blunt-nosed viper venom has been studied only recently. We investigate analgesic and anti-inflammatory effects of a combination of essential oil and snake venom.

Methods. In a study on white mice, a formalin, the "acetic acid writhes" and "Hot plate" tests were carried out, and the presence of cytotoxic action of these substances in the culture of normal and cancer cells was investigated using the MTT assay test.

Results. In the formalin test, 4% OVA essential oil solution (3.5 mg/mouse) exerts significant antinociceptive and anti-inflammatory effects ($p=0.003$). MTT assay shows approximately 60% cytotoxicity in HeLa and Vero cells for 2.0 μL/mL OVA essential oil in media. Statistically reliable data were obtained on the pronounced analgesic effects, both separately of essential oil of oregano and snake venoms (five species of cobras and blunt-nosed viper). The combination of venom and essential oil shows a more pronounced analgesic effect (up to 80%, $p<0.05$).

Conclusion. The selection of therapeutic doses (0.1 -0.03 of LD50) of both essential oil and venoms made it possible to obtain a combined preparation with high analgesic and inflammatory activity. The drug acts both on the cannabinoid system of the organism, and on the conduction of afferent impulses and central structures involved in the regulation of pain. The combination of oregano essential oil with a high content of beta-caryophyllene and snake venom may be chosen for further development of pain relief remedy.

ASSOCIATION WITH END-BINDING PROTEINS CONTROLS STIM2 PROTEIN CLUSTERING

Rakovskaya A.V.[1], Pchitskaya E.I.[1], Bezprozvanny I.B.[1,2]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - UT Southwestern Medical Center at Dallas, Department of Physiology, Dallas, TX 75390, USA

jonatepl@gmail.com

Neuronal store-operated calcium entry in hippocampal neurons is regulated by the STIM2 protein. This pathway is disrupted in various Alzheimer's disease (AD) mouse models. Recent studies have shown that dynamic microtubules plus-ends covered by end-binding protein 3 (EB3) transiently enter neuronal dendritic spines. STIM2 forms a Ca2+-dependent complex with EB3 via the Ser-x-Ile-Pro amino acid motif. In hippocampal neurons association of these proteins is important for normal dendritic spines morphology [1]. During store depletion STIM proteins form clusters as a part of calcium entry initiation mechanisms. It is known that clusterization of homologues protein STIM1 is controlled by EB1. The aim of this work is to analyze the size and the density of STIM2 and STIM2-IP/NN with mutation in EB proteins interaction site in HEK293T cells. To detect the localization of protein clusters, it is necessary to obtain images with high resolution. An increase in resolution can be achieved using the expansion microscopy method. HEK293T cells with 50–70% of confluency were transfected with plasmids YFP-STIM2 and YFP-STIM2-IP/NN. Fixed cells were stained with primary antibodies to the GFP protein and secondary antibodies conjugated with the Alexa Fluor 488 fluorophore to enhance the luminescence intensity. To ensure cross-linking of the protein molecules of the sample with the gel, the cells were treated with Acryloyl-X and gel-embedded. After treatment with proteinase K, the gel was successively expanded in water 3 times. We found that the distance between the clusters is smaller for the STIM2-IP/NN protein in comparison to STIM2 and their density is lower. Therefore, we may conclude that clustering of STIM2 protein is controlled by dynamic microtubules.

1. Pchitskaya, E., Kraskovskaya, N., Chernyuk, D. et al. Stim2-Eb3 Association and Morphology of Dendritic Spines in Hippocampal Neurons. Sci Rep 7, 17625 (2017).

Supported by RNF № 21-74-00028.

ASSOCIATION WITH PROTEASOME DETERMINES PATHOGENIC THRESHOLD OF POLYGLUTAMINE EXPANSION DISEASES

Kim M., Bezprozvanny I.

UT Southwestern Medical Center, Dallas, USA

meewhi.kim@utsouthwestern.edu

Expansion of glutamine residue track (polyQ) within soluble protein is responsible for eight autosomal-dominant genetic neurodegenerative disorders. These disorders affect cerebellum, striatum, basal ganglia and other brain regions. Each disease develops when polyQ expansion exceeds a pathogenic threshold (Qth). A pathogenic threshold is unique for each disease but the reasons for variability in Qth within this family of proteins are poorly understood. In the previous publication we proposed that polarity of the regions flanking polyQ track in each protein plays a key role in defining Qth value. To explain the correlation between the polarity of the flanking sequences and Qth we performed quantitative analysis of interactions between polyQ-expanded proteins and proteasome. Based on structural and theoretical modeling, we predict that Qth value is determined by the energy of polar interaction of the flanking regions with the polyQ and proteasome. More polar flanking regions facilitate unfolding of α-helical polyQ conformation adopted inside the proteasome and as a result, increase Qth. Predictions of our model are consistent with Qth values observed in clinic for each of the eight polyQ-expansion disorders. Our results suggest that the agents that can destabilize polyQ α-helical structure may have a beneficial therapeutic effect for treatment of polyQ-expansion disorders.

Literature

1. Kim, M.W., Y. Chelliah, S.W. Kim, Z. Otwinowski, and I. Bezprozvanny. (2009) Secondary structure of Huntingtin amino-terminal region. Structure. 17(9): p. 1205-12.

2. Kim, M. (2013) Beta conformation of polyglutamine track revealed by a crystal structure of Huntingtin N-terminal region with insertion of three histidine residues. Prion. 7(3): p. 221-8.

3. Kim, M. (2014) Pathogenic polyglutamine expansion length correlates with polarity of the flanking sequences. Mol Neurodegener. 9(1): p. 45.

4. Kim, M. and I. Bezprozvanny. (2021) Association with proteasome determines pathogenic threshold of polyglutamine expansion diseases. Biochem Biophys Res Commun. 536: p. 95-99.

Supported by: Russian Science Foundation Grant 19-15-00184 (IB) and by the National Institutes of Health grant R01NS056224 (IB).

ASTROCYTIC CALCIUM CORRELATES OF ANIMAL BEHAVIOR

Fedotova A.[1,2], Brazhe A.[1,2], Tiaglik A.[1,2], Denisov P.[1], Toptunov D.[3], Pryazhnikov E.[3], Khiroug L.[3], Fedotov I.[2], Solotenkov M.[2], Zheltikov A.[2], Semyanov A.[1,2]

1 - Institute of Bioorganic Chemistry RAS, Moscow, Russia
2 - Moscow State University, Moscow, Russia
3 - Neurotar Helsinki, Helsinki, Finland

semyanov@ibch.ru

Electrode based techniques have long been used to record neuronal activity in the brain. They revealed specific patterns of neuron firing associated with different forms of animal behaviour. Such discoveries led to the paradigm that brain function depends on the organization of neuronal networks and the plasticity of synaptic connections. Other cell types in the brain were considered supportive to neurons. However, the research of past decades has uncovered the role of non-neuronal cells in brain function. The concept of brain active milieu describes functional interactions among neurons, neuroglia, blood vessels and dynamical extracellular space as a basis of brain functions (Semyanov&Verkhratsky 2022). This concept provides a framework for experimental research in vivo. Within this framework, we asked how specific spatiotemporal patterns of astrocytic calcium activity can be related to mouse behaviour. Firstly, we defined the parameters of populational calcium activity in hippocampal slices. We found that fluctuations in areas but not in the density of astrocytic regions of activity (ROA) define changes in the overall activity area. Then we performed two-photon imaging of astrocytes in the somatosensory cortex in awake head-fixed mice on an air-lifted platform. We found that enlargement of ROA correlates with an initial phase of animal running, while the density of ROA correlates with animal speed. Finally, we performed calcium imaging in freely moving mice with optic fibre photometry. We discovered that calcium activity in hippocampal astrocytes is specific to animal behaviour in various behavioural tests. Thus, we conclude that similar to neuronal firing, changes in astrocytic calcium activity are specific to animal behaviour. This discovery supports the main postulate of the brain active milieu concept that brain functions are defined by orchestrated response of neuronal and non-neuronal elements. It also suggests that non-neuronal elements should be considered for brain activity readout in brain-computer interfaces.

Supported by Russian Science Foundation grant 20-14-00241

BCL-2 PROTEIN CLUSTERS FORM CONTACTS WITH IP3 RECEPTOR CLUSTERS IN MOUSE HIPPOCAMPAL NEURONS IN VIVO

Chigray M.[1], Rakovskaya A.[1], Pchitskaya E.[1], Vervliet T.[2], Bultynck G.[2], Bezprozvanny I.[1,3]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Laboratory of Molecular & Cellular Signaling, KU Leuven, Leuven, Belgium
3 - Department of Physiology, UT Southwestern Medical Center at Dallas, Dallas, TX 75390, USA

Ilya.Bezprozvanny@UTSouthwestern.edu

Bcl-2-family members have emerging as critically modulators of intracellular Ca^{2+} dynamics. The founding member, anti-apoptotic Bcl-2 inhibits Ca^{2+} release from the endoplasmic reticulum (ER) by directly binding and inhibiting inositol 1,4,5-trisphosphate receptors (IP3Rs), intracellular Ca^{2+}-release channel. The binding of Bcl-2 to the IP3R suppresses pro-apoptotic Ca^{2+} signaling, indicating that the Bcl-2-IP3R complex is a potential therapeutic target for diseases associated with cell-death resistance due to Bcl-2, such as certain cancers. The BH4 domain of the Bcl-2 protein is required and sufficient for binding to and inhibiting the IP3R. Mutating K17 into D (yielding Bcl-2K17D protein) alleviates Bcl-2's ability to potently bind and inhibit IP3R channels.

In the current experiments, we set out to assess the co-localization of Bcl-2 and the IP3R in neuronal cell systems. Both proteins were found to reside in the soma of neurons, mainly in the form of clusters that are characterized by a low level of co-localization in both wild-type hippocampal neurons and 5xFAD line. The application of the expansion microscopy method made it possible to demonstrate that some Bcl-2 clusters are in contact with IP3R1 clusters. Based on the data obtained, it was suggested that these proteins are located in different cellular organelles, IP3R1 is located in the ER, and Bcl-2 in the mitochondria, where it clusters at the sites of their contact with the ER, the so-called mitochondria associated membranes (MAMs). It was found that, similarly to mCherry-Bcl-2, the mCherry-Bcl2-K17D protein, which is impaired in IP3R binding, also forms clusters in the soma of hippocampal neurons.

Supported by RNF № 20-45-01004.

BIOSYNTHESIS OF SEROTONIN UNDER DIET-INDUCED OBESITY IN MICE

Mikhailova E.V., Avrova D.K., Romanova I.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

elenamikhailova87@gmail.com

Serotonin is one of the most important neurotransmitters in the brain, which is involved in the control of food intake. In the brain the raphe nuclei (RN) neurons of the midbrain are the main source of serotonin; they express tryptophan hydroxylase-2 (TPH2) – a key enzyme in serotonin biosynthesis. Projections from RN enter the nuclei of the hypothalamus, in particular, to the arcuate nucleus (ARC) neurons, which control eating behavior and the energy balance. In ARC neurons the expression of serotonin 5-HT_{1B} and 5-HT_{2C} receptors was revealed, which changes in obesity. In C57Bl/6J mice, after 16 weeks keeping on a high-calorie diet, diet-induced obesity (DIO) developed, and a decrease in the TPH2 level was detected immunohistochemically in dorsal RN (dRN) neurons. The aim of the study - to compare the level of *Tph2* gene expression in the midbrain and in the hypothalamus, as well as to evaluate the mechanisms aimed at control of serotonin level in the brain in DIO. By real-time PCR in DIO was shown a decrease in the TPH2 mRNA level in the midbrain ($p<0.05$) and no changes in the hypothalamus, which indicates the existence of additional sources of serotonin biosynthesis. By double immunofluorescence method in DIO in 5-HT- neurons of dRN an increase in the level of phospho(serine-19)TPH2 ($p<0.05$) and an increase in the level of the neurotrophic factor BDNF ($p<0.05$) were detected. The received data demonstrates the possibility of TPH2 expression in hypothalamic neurons, which, obviously, is aimed at increasing brain serotonin in obesity. The results of analysis of *Akt1*-kinase expression and its phosphorylation (phospho-serine-473 Akt1) in the hypothalamus and midbrain are presented.

Thus, in DIO activates compensatory mechanisms aimed at maintaining the viability of serotonergic neurons and their functional activity.

The study was supported by state budget.

BCL-2 PROTEIN PREVENTS SYNAPTIC LOSS IN 5×FAD MOUSE MODEL OF ALZHEIMER'S DISEASE

Chernyuk D.[1], Vervliet T.[2], Gordeev A.[1], Polozova M.[1], Pchitskaya E.[1], Bultynck G.[2], Bezprozvanny I.[1,3]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Laboratory of Molecular & Cellular Signaling, KU Leuven, Leuven, Belgium
3 - Department of Physiology, UT Southwestern Medical Center at Dallas, Dallas, TX, USA

Ilya.Bezprozvanny@UTSouthwestern.edu.

Alzheimer's disease (AD) is the most common neurodegenerative disorder. A proximal feature of the pathogenesis of AD is a dysregulation of intracellular calcium (Ca^{2+}) signaling in neurons, in particular enhanced Ca^{2+} release from endoplasmic reticulum located Ca^{2+} release channels including $InsP_3R1$ and RyanR2. Our own and other studies demonstrated that Bcl-2 proteins inhibit the activity of the $InsP_3R1$ and RyanR2. In the present study, we evaluated the hypothesis that elevating Bcl-2-protein levels is able to normalize neuronal Ca^{2+} and exert neuroprotective effects in an AD mouse model. To test this hypothesis, we used adeno-associated viruses (AAV) to express Bcl-2 proteins in hippocampal region of 5xFAD mouse model of AD. In our studies, 5xFAD mice was crossed with line M mice that expresses GFP in selected neuronal population. To specifically evaluate the importance of association with $InsP_3R$, a Bcl-2^{K17D} mutant was also expressed via AAV. This K17D mutation was previously demonstrated to diminish the association of Bcl-2 with the $InsP_3R1$ thereby alleviating its ability to inhibit $InsP_3Rs$.

In our experiments, we discovered that overexpression of both wild-type Bcl-2 and Bcl-2^{K17D} mutant resulted in rescue of mushroom spine loss in 5xFAD mice. Thus, we concluded that expression of Bcl-2 protein results in synapto-protective effects in AD animal model, but this effect is not due to Bcl-2-mediated inhibition of $InsP_3R1$ channels. Potential mechanisms of this synapto-protective effects of Bcl-2 may be related to its ability to inhibit RyanR2 activity, as our previous studies indicated that Bcl-2^{K17D} mutant remains capable of binding and inhibiting RyanR2 channels. It is also possible that Bcl-2 exerts neuroprotective effects independently of its modulation of Ca^{2+} signaling, but via its canonical anti-apoptotic properties. Further experiments are needed to establish the mechanisms underlying the neuroprotective effects of Bcl-2 in AD models.

Supported by RNF No. 20-45-01004.

CENTRAL INJECTION OF THE CEREBRAL DOPAMINE NEUROTROPHIC FACTOR (CDNF) AFFECTED BEHAVIOR AND ENHANSED SEROTONIN TURNOVER IN BRAIN OF MICE

Tsybko A.S., Eremin D.V., Khotskin N.V., Ilchibaeva T.V., Naumenko V.S.

Federal research center Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia

antoncybko@mail.ru

Cerebral dopamine neurotrophic factor (CDNF) is considered to be a protective factor for brain dopaminergic neurons. A little is known about the involvement of CDNF in the regulation of behavior aside from locomotor activity as well as modulation of other neurotransmitter systems like serotonin (5-HT) system. To address this question the effects of central (i.c.v.) administration of the recombinant CDNF protein on behavior and 5-HT turnover in the brain of mice were investigated. Males of C56Bl6/J mice were acutely treated with recombinant human CDNF protein (3, 10 or 30 μg i.c.v.) or PBS. Home-cage behavior was assessed for 3 days after injection using PhenoMaster system. Anxiety, exploratory and depressive-like behavior was assessed after CDNF i.c.v. injection in additional experiment. CDNF in all used dosage failed to produce any significant changes in the locomotor activity, food and water consumption. Only sleep duration in first 24 h of testing was affected by injection of 3 μg of CDNF. Notably, CDNF in all dosage significantly improved associative learning assessed in operant-wall test. In the dosage 10 and 30 μg the CDNF produced marked anxiolytic and anti-depressant effects as well as increased exploratory activity. The 5-HT turnover was increased in all studied brain structures of mice treated with CDNF in all dosage. Simultaneously, we have revealed significant changes in the mRNA level of the genes responsible for reception (*Htr1a, Htr7*) and catabolism (*Maoa*) of 5-HT in the frontal cortex, hippocampus and hypothalamus of animals treated with CDNF.

Thus, we have shown for the first time that CDNF when injected i.c.v. can ameliorate the behavior of animals. These pro-cognitive, anxiolytic and anti-depressant effects could be linked, at least partially, with enhanced 5-HT turnover produced by CDNF injection.

The work was supported by the Russian Science Foundation (grant No. 22-15-00011) and basic research project No. FWNR-2022-0023.

CAN NGF PROTECT AGAINST BEHAVIORAL IMPAIRMENTS CAUSED BY HIPPOCAMPAL CHOLINERGIC DEFICIT?

Bolshakov A.P., Dobryakova Y.S., Stepanichev M.Yu., Spivak Yu.S., Zaichenko M.I., Koryagina A.A., Markevich V.A.

Institute of Higher Nervous Activity and Neurophysiology, Moscow, Russia

al230679@yandex.ru

Development of cognitive impairments in Alzheimer's disease is associated with loss of cholinergic neurons in the basal forebrain, including the medial septal area. In our studies, we used a model of hippocampal cholinergic deficit, which results from degeneration of septal cholinergic neurons caused by immunotoxin 192IgG-saporin, to study possible beneficial effects of overexpression of nerve growth factor (NGF) in the hippocampus of rats with cholinergic deficit. To overexpress NGF, we used a suspension of recombinant viruses carrying cassette with NGF which was injected into both hippocampi of rats with hippocampal cholinergic deficit induced by injection of 192IgG-saporin. Induction of cholinergic deficit in the hippocampus impaired behavioral performance in Y-maze and beam-walking test but did not affect behavioral indices in the T-maze, open field test, and inhibitory avoidance training. NGF overexpression in the rats with cholinergic deficit restored animal performance in Y-maze and beam-walking test. In addition to behavioral performance, NGF also improved synaptic plasticity in the hippocampus. Recording of field excitatory postsynaptic potentials in vivo in the hippocampal CA1 area showed that NGF overexpression reversed suppression of long-term potentiation that appeared after induction of cholinergic deficit. The beneficial effect of NGF was not related to compensatory changes in the expression of NGF receptors TrkA and NGFR in the hippocampus and medial septal area. NGF overexpression also did not prevent a 192IgG-saporin–induced decrease in the activity of acetylcholine esterase in the hippocampus and strong loss of cholinergic neurons in the septal area caused by the immunotoxin. We conclude that NGF overexpression in the hippocampus under conditions of cholinergic deficit induces beneficial effects which are not related to maintenance of cholinergic function.

CELL MODEL FOR INVESTIGATING 14-3-3 PROTEIN INTERACTOME IN THE HUNTINGTON'S DISEASE

Makeeva V.S., Malakhova A.A., Malankhanova T.V., Medvedev S.P., Zakian S.M.

Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

vladakonyukhova@gmail.com

Huntington's disease (HD) is a neurodegenerative autosomal dominant monogenic disorder. It is induced by the expansion of CAG trinucleotide in the first exon of the HTT gene, which provides mutant huntingtin protein (mHTT) with an abnormal conformation. The expression of mHTT results in the death of spiny neurons of the striatum of the brain.

Molecular mechanisms of the development of this disease are not known for certain, as the role of mutant huntingtin in pathogenesis is not clear.

14-3-3 proteins were found to play a protective role in neurodegeneration. 14-3-3 is an evolutionarily conserved protein family that in mammals consists of seven isoforms. These proteins regulate most cell processes and maintain cell viability. Some isoforms of 14-3-3 interact with mutant huntingtin, participating in the aggresome formation reaction.

Here, we present an approach for studying the mechanisms of Huntington's disease. It is based on an isogenic cell model, that includes the HEK293FT-B5 cell line generated from HEK293FT cells to express mHTT, which reproduces the phenotype of Huntington's disease. Both HEK293FT and HEK293FT-B5 cells express transgenic 14-3-3 protein subunits with the tags for tandem affinity purification.

We use the transgenic cell lines to purify the protein complexes and identify the compounds of the 14-3-3 interactomes. We expect to find the changes in the protein-protein interaction pattern under the influence of the mHTT expression.

CHANGES IN GLUTAMATERGIC AND GABAERGIC SYSTEMS DURING DEVELOPMENT OF ALZHEIMER'S DISEASE-LIKE PATHOLOGY IN OXYS RATS

Burnyasheva A.O., Rudnitskaya E.A., Telegina D.V.

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

burn.alena2505@mail.ru

Alzheimer's disease (AD) is an incurable neurodegenerative disorder. Dysregulation of the balance of glutamatergic and GABAergic systems accompany AD development. However, the question concerning this dysregulation being the cause or the consequence of AD development remains unanswered. Using senescence-accelerated OXYS rats as a suitable model of AD, we assessed the relationship between changes in glutamate and GABA systems in the hippocampus and the development of AD signs.

The content of enzymes that regulate the glutamate-GABA cycle–glutaminase (GLS), glutamate decarboxylase (GAD67), glutamine synthetase (GS) and GABA-transaminase–was analyzed in the hippocampus of OXYS rats at different stages of the AD sign development (1.5, 4, 12 and 18 months of age) and in Wistar rats (control) by Western blot and immunohistochemical analyses. Additionally, the content of NMDA receptor 1 (NMDAR1) was evaluated.

We did not observe significant age-related as well as interstrain changes in the levels of GLS and GS. Meanwhile, the level of GABA-transaminase was lower and GAD67 was higher in OXYS rats throughout the lifespan. This result is in line with the observation of our colleagues about higher level of GABA in the hippocampus of OXYS rats compared to Wistar rats. The level of NMDAR1 decreased with age in the hippocampus of Wistar rats; however, we did not observe this decrease in OXYS rats. As a result, NMDAR1 level was higher in OXYS rats at 18 months of age.

Development of AD signs in OXYS rats occurs against background of altered balance of the glutamatergic and GABAergic systems with shift towards higher GABA levels which may contribute to the progression of neurodegeneration. Increased NMDAR1 content in the hippocampus of OXYS rats at 18 months of age together with higher GABA levels points to hyperactivation of both neurotransmitter systems at progressive stage of AD-like pathology.

Supported by RSF (project #21-75-00029).

CHANGES IN NEPRILYSIN EXPRESSION AND DISTRIBUTION IN FOREBRAIN STRUCTURES CORRELATE WITH IMPAIRMENT OF OLFACTORY AND COGNITIVE FUNCTIONS IN 5XFAD MICE

Vasilev D.S., Dubrovskaya N.M., Tumanova N.L., Nalivaeva N.N.

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, RAS, St. Petersburg, Russia

dvasilyev@bk.ru

Neprilysin (NEP) is an important proteolytic enzyme terminating action of various biologically active peptides including amyloid (Aβ) peptide preventing its accumulation which is causative of Alzheimer's disease (AD). Although NEP functions have been studied in animal models, little is known about its expression and distribution in different brain regions and regulation of its expression. Using wild-type (WT) and 5xFAD transgenic mice we analyzed the dynamics of NEP mRNA expression by rtPCR in the olfactory bulbs (OB), parietal (PCx), pyriform (PyrCx) and entorhinal (ECx) cortices, hippocampus (Hip) and striatum (Str), and analyzed mouse behavior in the Morris water maze, odor preference and food search tests. The data demonstrate that NEP mRNA expression increases during the first postnatal month being the highest in OB and Str. By the age of three months, in both WT and 5xFAD mice, NEP mRNA levels sharply decrease in PCx, PyrCx, ECx and Hip, and by nine months in OB. In PCx, PyrCx, ECx and Hip of 5xFAD mice NEP expression was lower than in WT mice at all ages analyzed. Immunohistochemical labeling showed altered NEP distribution in the cortical brain areas of the 5xFAD mice with a significant decrease of NEP protein levels in the cell bodies of cortical neurons compared to WT mice. In 5xFAD mice decreased NEP expression correlated with accumulation of amyloid plaques, impaired olfaction and memory. Administration of an HDAC inhibitor valproic acid restored NEP expression in brain tissue of 5xFAD mice and improved their olfactory and cognitive function. Our data testify to the important role of NEP in olfaction and confirm the validity of pharmacological up-regulation of NEP expression leading to improved behavior in olfactory and cognitive tests. This opens avenues for NEP-based pharmacological approaches to treat AD-related cognitive decline and impaired olfaction.

Supported by Russian state budget assignment 075-00408-21-00.

CHOLINE ACETYLTRANSFERASE GENE INACTIVATION USING THE CRISPR-CAS9 SYSTEM

Koryagina A.A.[1], Spivak Yu.S.[1], Bolshakov A.P.[1], Dashinimaev E.B.[2], Gulyaeva N.V.[1], Stepanichev M.Yu.[1]

1 - Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia
2 - Kol'tsov Institute of Developmental Biology, RAS, Moscow, Russia

koraljona3@gmail.com

Forebrain cholinergic neurons modulate rhythmic activity in the neocortex and hippocampus and have an impact on memory formation and consolidation. Previously, the role of cholinergic neurons was studied using various toxins that lead to damage and death of neurons. Recent data show that forebrain cholinergic neurons are able to synthesize and to release both acetylcholine (ACh) and gamma-aminobutyric acid. In this case previously used approaches do not allow to differentiate the functional role of each of two neurotransmitters. To shed light upon the contribution of individual neurotransmitters to brain function it is necessary to develop new approaches for inactivation of one neurotransmitter with no effect on another one. Up-to-date genome engineering technologies such as CRISPR-Cas9 can be used for precise gene editing in mammalian neurons *in vivo* resulting in frame-shifting insertion/deletion mutations and subsequent protein depletion.

Choline acetyltransferase (ChAT) is the main enzyme catalyzing ACh synthesis from choline and acetyl-CoA in cholinergic neurons. In this study, we developed a system for local inhibition of ACh transmission between medial septum and hippocampus based on application of CRISPR-Cas9 to knock down the expression of the *Chat* gene. We prepared a series of serotype 9 adeno-associated viral (AAV9)-based vectors carrying SpCas9 and guide RNAs to target the *Chat* gene. This system allowed deletion in the target gene in neuroblastoma NB41A3 culture cells. Administration of the vectors in the medial septum of the adult mouse brain resulted in a decrease in ChAT activity in the hippocampus. We believe that this system of selective inhibition of ACh neurotransmission will make it possible to preserve structural and functional properties of neurons. Additional studies are necessary to further characterize the effects of this inhibition in neurons using biochemical, genetic, electrophysiological and behavioral readouts.

Supported by the RFBR (project #20-015-00226).

CHRONIC SLEEP RESTRICTION LEADS TO THE FUNCTIONAL DISTURBANCES IN MONOAMINERGIC SYSTEMS OF THE BRAIN AND ENDOCRINE DYSFUNCTIONS IN RATS

Lapshina K.V., Ekimova I.V., Pazi M.B., Belan D.V., Matvevnina D.N., Derkach K.V., Shpakov A.O.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St.Petersburg, Russia

ksenia.lapshina@gmail.com

Clinical and experimental studies demonstrate that habitual short sleep duration predisposes to the development of metabolic and cardiovascular diseases, affective disorders, erectile dysfunction. However, there is no overall concept explaining the reasons for these disturbances. The data on the severity of sleep loss-induced damage in various brain structures including the hypothalamus, which is responsible for the regulation of the endocrine system, remain insufficient. The study was aimed to assess destructive changes in the monoaminergic structures of the brain stem and disorders of the hormonal and metabolic status caused by the chronic sleep restriction (CSR) in rats.

A model of CSR was studied in six-month-old male Wistar rats which underwent cycles of 3 h of SR and 1 h of sleep opportunity continuously for 5 days on the programmed orbital shaker.

After day 5 of CSR, acute and delayed damaging effects were found in the ventral tegmental area (VTA) and in the locus coeruleus (LC), which mediate the regulation of the sleep-wake cycle, endocrine-metabolic, autonomic and behavioral responses of the body. The number of tyrosine hydroxylase-immunopositive and Nissl-stained neurons was decreased by 22% in the VTA and by 18% in the LC. The CSR-induced apoptosis and neuronal death were due to activation of endoplasmic reticulum stress (GRP78/PERK/CHOP cascade) and caspases-3 and -9. During CSR we revealed the decrease in appetite and body weight, levels of insulin, leptin and testosterone and the significant increase in the level of corticosterone in the blood serum. There was no progression of neurodegeneration and marked hormonal changes 56 days after the end of CSR. The data obtained are important for understanding the mechanisms of development of neuropsychiatric, metabolic and endocrine disorders in CSR.

Supported by the Ministry of Science and Higher Education of the Russian Federation grant (№ 075-15-2020-916 dated November 13, 2020).

COMPARATIVE ANALYSIS OF THE OREXIN-IMMUNOPOSITIVE STRUCTURES IN THE HYPOTHALAMUS AND OLFACTORY EPITHELIUM OF RAT EMBRYOS

Morina I.Yu., Mikhailova E.V., Romanova I.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St.Petersburg, Russia

irinamorina@mail.ru

Orexins (A and B) were first identified in the brain neurons of the lateral hypothalamus. Their functions are associated with the regulation of the energy balance, food intake, wakefulness, of the immune response, etc. via their effects on ORX1 or ORX2 receptors. During rat embryogenesis (E) orexin-producing neurons in the hypothalamus begin to appear after E13, and orexins (A and B) can play the role of morphogenetic factors. At present, the expression of orexins and their receptors has also been detected in other mammalian tissues, in particular, in the olfactory epithelium. The aim of this study was to assess the level of orexin-producing structures development in the hypothalamus and olfactory epithelium of rat embryos (E18). Real-time PCR was used to analyze the mRNA levels of pre-proorexin – precursor of orexins A and B, ORX1 or ORX2 receptors in the hypothalamus and the olfactory epithelium. By single immunolabeling immunopositive to orexin-A and orexin-B structures in these regions was detected. By double fluorescent immunolabeling and a confocal microscope we analyzed various mediatory systems which are involved in regulation of orexin-containing structures in hypothalamus and olfactory epithelium. The obtained data indicate the advanced development of orexin-containing structures in the peripheral nervous system compared to the hypothalamic and are discussed in connection with the possibility of morphogenetic role of peripheral orexins on CNS. Some comparative aspects of the distribution of orexins in the olfactory epithelium of lower vertebrates will be discussed. *The study was supported by state budget.*

COMPARATIVE STUDY OF LEARNING IN THE MORRIS WATER MAZE TEST OF ALCOHOL-NAIVE AND LONG-TERM ETHANOL-EXPERIENCED WISTAR RATS AND HETEROZYGOUS DOPAMINE TRANSPORTER KNOCKOUT RATS

Gromova G.[1,2], Dorofeikova M.[1], Filatova E.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia
2 - Herzen University, St. Petersburg, Russia

411225@rambler.ru

Chronic ethanol exposure enhances dopamine neurotransmission and, as a result, increases the levels of the dopamine transporter (DAT) in several brain regions. We used intermittent ethanol access and heterozygous DAT knockout (DAT-HET) rats to model dopaminergic system dysfunctions and assessed learning and memory.

Adult male Wistar (10 alcohol-naive and 10 that had intermittent access to alcohol for 4 months) and DAT-HET (n=14) rats were used. To evaluate learning and spatial memory, we used Morris water maze test. Rats were trained in two tasks: to find a visible platform (4 trials/day for 2 days) and to find an invisible platform which was placed beneath the water (4 trials/day for 3 days).

Significant differences were found in the number of failed trials during day 1, 3-5 ($p<0.001$; $p=0.008$; $p=0.037$; $p=0.006$), DAT-HET animals failing the most. Perhaps it is associated with a tendency DAT-HET rats to remain close the walls (thigmotaxis). Average time spent near the walls was 11.1±3.3 s for alcohol-naive Wistar rats, 16.6±2.3 s for alcohol-consuming Wistar rats, and 43.7±2.4 s for DAT-HET during day 1, and 3.2±1.8 s, 2.0±1.8 s, 11.1±1.5 s during day 5. Significant differences were found in the time of the thigmotaxis during all days (day 1: $p<0.001$; day 2: $p=0,011$; day 3: $p=0,021$; day 4: $p<0,001$ day 5: $p=0.024$). Escape latency was also longer in DAT-HET rats during days 1, 2 and 4 (day 1: $p=0.053$; day 2: $p<0,001$; day 4: $p=0,003$).

Overall, DAT-HET rats exhibited significantly impaired learning and spatial memory, as well as unconstructive thigmotaxis that has been demonstrated previously as an effect of increased dopaminergic transmission (Simon et al, 1994), compared to alcohol-naïve and alcohol-consuming Wistar rats.

This work was supported by St. Petersburg State University that provided DAT-HET rats and Russian Federal Agency for Scientific Organizations (research theme № AAAA-A18-118012290373-7).

COMPARISON OF THE DAMAGING EFFECTS OF PRENATAL AND POSTNATAL HYPERHOMOCYSTEINEMIA ON THE NERVOUS SYSTEM OF OFFSPRING

Milyutina Yu.P.[1], Shcherbitskaia A.D.[1,2], Zalozniaia I.V.[1], Arutjunyan A.V.[1]

1 - Ott Institute of Obstetrics, Gynecology, and Reproductive medicine, St. Petersburg, Russia
2 - Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

milyutina1010@mail.ru

Maternal hyperhomocysteinemia (HHC), is accompanied by generation of reactive oxygen species and is detected by an increased level of the serum L-homocysteine. HHC causes various complications in the pregnancy course that are based on placental dysfunction. HHC research in the context of neurodegeneration and cognitive abilities have gained more importance in recent years, however, little is known about the effects of prenatal HHC on subsequent brain development in adult animals. We investigated the influence of prenatal and postnatal HHC on biogenic amines content in hypothalamus and hippocampus of pubertal female rats. HHC was created by administration *per os* of 0.15% aqueous L-methionine solution (0.10-0.15g per animal) to pregnant or adult rats using long-term methionine loading. It was shown that high maternal homocysteine in pregnancy induces oxidative stress and apoptosis in the brains of newborn pups and phosphorylation p38 MAPK in the hippocampus of offspring brain in early ontogenesis, which can lead to delaying development of nerve tissue and weakening of different brain functions. The decrease of biogenic amines level in hippocampus after postnatal and prenatal HHC is accompanied by weakening of cognitive function in the pubertal female rats. HHC also induces the enhancement of the norepinephrine level in medial preoptic area of hypothalamus while prenatal HHC leads to its suppression. Prenatal HHC elevates the dopamine content in median eminence with the arcuate nuclei of hypothalamus, while estrous cycles are not disturbed.

Both models of HHC impaired various types of memory in the pubertal female rats, which allows asserting that maternal HHC is one of the pathological factors that can disrupt the processes of fetal brain development and cause persistent long-term effects in various functional systems of the growing organism.

Supported by Russian state budget assignment 1021062812133-0-3.2.2.

CONTROLLED β-AMYLOIDOSIS: THE ROLE OF ZINC, isoAsp7-Aβ AND TETRAPEPTIDE HAEE

Mitkevich V.A., Barykin E.P., Eremina S.Y., Adzhubei A.A., Kozin S.A., Makarov A.A.

Engelhardt institute of molecular biology, Moscow, Russia

mitkevich@gmail.com

The pathogenesis of Alzheimer's disease (AD) is associated with the formation of cerebral amyloid plaques, the main components of which are the modified Aβ molecules as well as metal ions. Aβ isomerized at Asp7 residue (isoD7-Aβ) is the most abundant isoform in amyloid plaques. In contrast to intact Aβ, intravenously administered isoD7-Aβ sharply accelerates cerebral amyloidosis in transgenic mice overexpressing human Aβ, potentially acting as an amyloid seeding. Moreover, synthetic isoD7-Aβ, rather than intact Aβ, causes a significantly higher level of tau phosphorylation in cell culture. These findings suggest the role of isoD7-Aβ as a molecular trigger for the pathogenic cascade of AD and a potential drug target. We hypothesized that the pathogenic effect of isoD7-Aβ is due to the formation of zinc-dependent oligomers, and that this interaction can be disrupted by the rationally designed tetrapeptide (HAEE). We utilized *C. elegans* model of Aβ amyloidosis to study the effect of isoD7-Aβ and zinc ions on animal pathophysiology and aging. We show that the concurrent administration of Zn^{2+} and isoD7-Aβ leads to a significant increase in amyloidosis accompanied by the shortening of animals' lifespan. We further demonstrate that the tetrapeptide, HAEE, previously designed to counter the receptor toxicity of Aβ *in vitro* [13] , can prevent zinc-induced oligomerization of isoD7-Aβ, negate the pro-amyloid effects of isoD7-Aβ:Zn^{2+} in live animals, and restore their lifespan. Our SPR, NMR, and molecular dynamics (MD) studies indicate that the molecular mechanism underlying the anti-amyloid effect of HAEE relies on its specific zinc-dependent and stable binding to Aβ and isoD7-Aβ. Together, these results elucidate the fundamental role of non-covalent complexes between zinc ion and isoD7-Aβ in triggering the pathological aggregation of endogenous Aβ molecules and suggest that the compounds targeting such complexes have a therapeutic potential.

Supported by the Russian Science Foundation, grant #19-74-30007.

DISTURBANCES IN THE GLUCOCORTICOID AND SEROTONIN SYSTEM CROSS-TALKS ARE ASSOCIATED WITH A TENDENCY TO DEPRESSION IN RATS SURVIVED PRENATAL HYPOXIA

Vetrovoy O.V.[1,2], Stratilov V.A.[1], Tyulkova E.I.[1]

1 - Pavlov Institute of Physiology Russian Academy of Sciences, St. Petersburg, Russia
2 - Saint Petersburg State University, St. Petersburg, Russia

vov210292@yandex.ru

Prenatal hypoxia-related neuropathologies are predominantly associated with impaired maternal glucocorticoid stimulation of the fetus, that is "imprinted" in decreased sensitivity of glucocorticoid reception by extrahypothalamic brain structures of offspring during lifespan. This leads to disruption of the glucocorticoid negative feedback and excessive glucocorticoid stimulation of the recipient tissues. The aim of this study was to investigate the glucocorticoid and serotonin system cross-talks and the characteristics of the response to mild stress in adult rats survived prenatal hypoxia (PH) on embryonic days 14-16. In the Raphe Nuclei (RN) of intact PH rats total and nuclear levels of glucocorticoid receptors (GR) and GR-dependent transcription are increased in compare with control. Mild stress leads to increase of GR-dependent transcription of monoamine oxidase A in RN and decease in serotonin levels in RN and hippocampus of PH rats (but not in control). In the hypothalamus of PH rats basal and mild stress-induced corticotropin-releasing hormone levels are increased in compare with control. This indicates impair of the effective functioning of the glucocorticoid negative feedback mechanism from extrahypothalamic structures. Meantime, if in response to mild stress in pituitary of control rats both synthesis of POMC and release of ACTH into the blood are decreased, in PH animals this negative feedback mechanism is disturbed. This is accompanied with changes in behavior: in response to mild stress PH rats (but not in control) demonstrate a decrease in exploratory activity and an increase in anxiety. Thus, the maternal stress response to hypoxia causes a glucocorticoid-dependent impairment of the serotonin system in offspring's brain, which predetermines the tendency to manifest the depressive states.

DE-DIFFERENTIATION OF FOREBRAIN CHOLINERGIC NEURONS IS PRECEDED BY OXIDATIVE STRESS IN EXPERIMENTAL MODELS OF NEURODEGENERATION

Stepanichev M., Nedogreeva O., Lazareva N., Moiseeva Yu., Onufriev M., Gulyaeva N.

Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Moscow, Russia

m_stepanichev@ihna.ru

Aging-related neurodegenerative diseases are associated with degeneration of specific neuronal populations. In Alzheimer's disease, loss of cholinergic neuronal phenotype in the forebrain starts before neuronal death and is often considered as a first step of de-differentiation. However, it is not clear what factors initiate this specific degeneration and at which stage therapeutic interventions may still reverse neurodegenerative process and rescue functional capacities of neurons. In the present study, we examined whether oxidative stress, i.e. excessive generation of free radicals and/or insufficiency of antioxidant defense, may be involved in this phenomenon. Central administration of neurotoxic amyloid-beta(25-35) peptide to adult male rats induced delayed impairments of cognitive functions. In this model of AD, we revealed that first signs of memory decline were related to a decrease in the expression of choline acetyltransferase (ChAT), the key enzyme of acetylcholine synthesis, in neurons of the medial septal area whereas the number of NeuN-positive neurons did not significantly change. However, oxidative stress was mostly pronounced during the first week after amyloid-beta(25-35) administration and preceded the development of amnesia. In olfactory bulbectomized mice, which also exhibit some AD-like signs, a decrease in the portion of ChAT-containing neurons in the medial septal area was also associated with start of cognitive impairments and also preceded by development of oxidative stress. We assume that in these two models of neurodegeneration, oxidative stress may cause cholinergic disfunction rather than irreversible neuron damage. These data indicate that cholinergic neurons of the basal forebrain are very sensitive to oxidative stress because of specific features of intracellular metabolism related to acetylcholine synthesis. The development of such cholinergic dysfunction may be responsible for the appearance of early cognitive decline in Alzheimer's disease.

Supported by the Russian Foundation of Basic Research (project #20-15-00226).

DEEP BRAIN STIMULATION OF THE MEDIAL SEPTAL AREA CAN MODULATE GENE EXPRESSION IN THE HIPPOCAMPUS OF RATS UNDER URETHANE ANESTHESIA

Dobryakova Y.V., Karan A.A., Spivak Yu.S., Medvedeva T.M., Markevich V.A., Bolshakov A.P.

Institute of Higher Nervous Activity and Neurophysiology of RAS, Moscow, Russia

julkadobr@gmail.com

The medial septal area is the major source of cholinergic innervation of the hippocampus, which is critical for learning and memory formation. Previous studies showed that memory formation is associated with changes in the expression of immediate early genes suggesting that activation of medial septal neurons may induce changes in the gene expression. We studied the effects of stimulation of the medial septal area at two depths on the gene expression in the dorsal and ventral hippocampus. In our experiments, the rats under urethane anesthesia were implanted with recording electrode in the right hippocampus and stimulating electrode in the medial septal area at depth of either 3.5-4.5 mm (dorsal medial septum, dMS) or 6.5 mm (medial septal nucleus, MSN) from dura. After one-hour-long deep brain stimulation by trains of paired pulses, we collected ipsi- and contralateral dorsal and ventral hippocampi. Quantitative PCR showed that deep brain stimulation did not cause any changes in the intact contralateral dorsal and ventral hippocampi. A comparison of ipsi- and contralateral hippocampi in the control unstimulated animals showed that electrode implantation in the ipsilateral dorsal hippocampus led to a dramatic increase in the expression of immediate early genes (c-fos, Arc, egr1, npas4), neurotrophins (ngf, bdnf) and inflammatory cytokines (il1b and tnf, but no il6) not only in the implantation site but also in the ventral hippocampus. Moreover, stimulation of MSN but not dMS further increased expression of c-fos, egr1, npas4, bdnf, and tnf in the ipsilateral ventral but not dorsal hippocampus. Our data suggest that activation of medial septal nucleus can change the gene expression in ventral hippocampal cells after their priming by other stimuli.

The study was supported by the Russian Science Foundation 22-25-00479.

DEVELOPMENT OF A CELL MODEL OF GBA ASSOCIATED PARKINSON'S DISEASE FOR IN VITRO DRUG TESTING

Drozdova E.[1,2], Grigor'eva E.[1], Pavlova S.[1], Malakhova A.[1], Sorogina D.[1,2], Ibatullin F.[3], Emelyanov A.[3], Pchelina S.[3], Medvedev S.[1], Zakian S.[1]

1 - Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia
2 - Novosibirsk State University, Novosibirsk, Russia
3 - NRC «Kurchatov Institute» - PNPI, Gatchina, Russia

e.drozdova2@g.nsu.ru

Induced pluripotent stem cells (iPSCs) are often used to create cell models because of their capability to self-renewal and differentiation into many cell types. Parkinson's disease (PD) is a neurodegenerative disorder that affects dopaminergic (DA) neurons in the brain. Heterozygous mutations in the *GBA1* gene are one of the disease causes. The mutations reduce the activity of glucocerebrosidase (GCase). This leads to the formation of Lewy bodies and the disease progression. Pharmacological chaperones (PCs) with a therapeutic effect can be suggested to increase the activity of GCase, such as ambroxol and NCGC00241607. The iPSC-based cell model is a perfect asset for *in vitro* drug testing.

Methods: Mononuclear cells of two patients with N370S mutation in the *GBA1* gene (a PD patient and an asymptomatic carrier) were reprogrammed to pluripotent state. The iPSC lines were characterized by karyotyping, immunofluorescent analysis and qPCR on pluripotency markers, and tested for spontaneous differentiation. Directed differentiation of iPSCs into DA neurons was carried out. The efficiency of the differentiation was assessed using immunofluorescence staining for specific markers (TH, LMX1A). The resulting neurons were cultured in the presence of PCs (50 μM ambroxol and 4 μM NCGC00241607), and the GCase activity was estimated.

Results: Three iPSC lines from each patient were standard characterized (Grigor'eva EV et al.,StemCellRes.2022.V.59.102651). The efficiency of DA neuron differentiation was analyzed by immunofluorescent staining. We showed a decrease of GCase activity in *GBA1*-DA neurons derived from iPSCs of both patients compared to control DA neurons. The GCase activity is increased in *GBA1*-DA neurons in the presence of PCs.

Conclusions: We have created a cell model of GBA-associated PD based on DA neurons obtained by directed differentiation of patient-specific iPSCs. The cell model can be used for drug screening.

The study was supported by the RSF grant No. 19-75-20063.

DEVELOPMENT OF CORTICAL SPREADING DEPRESSION IN SOMATOSENSORY CORTEX OF RAT WITH PRENATAL HYPERHOMOCYSTEINEMIA

Yakovlev A., Shakhamova V., Sitdikova G.

Kazan Federal University, Kazan, Russia

alv.yakovlev@gmail.com

Homocysteine is a sulfur-containing amino acid formed during the metabolism of methionine, in high concentrations with neurotoxic effects. Genetic mutations in metabolism enzymes, a deficiency of B vitamins leads to an increase in the level of homocysteine - hyperhomocysteinemia (hHCY). It was shown that hHCY participated in the damage and activation of endothelial cells of blood vessels, causes an increase in the permeability of the blood-brain barrier and, as a result, neurodegeneration. Cortical spreading depression (SD) is the underlying phenomenon of migraine headaches with aura and the incidence of which is associated with hHCY. The aim of study was investigating the sensitivity of the somatosensory cortex in the slices of rats with prenatal hHCY to SD generation. Experiments were performed on thalamocortical slices of the rat brain during third postnatal weeks. Extracellular local field potential was recorded the barrel cortex using glass pipette electrodes when filled with ACSF. SD-like waves were induced by 50 mM KCl puff application. Pups with prenatal hHCY were born from females received daily methionine with food. In control conditions negative deflections of DC potential shifts appeared in IV layers of somatosensory cortex with a time offset 2.3±0.4 s with amplitude was 0.4±0.05 mV and duration -3.5±0.5 s, (n=14), respectively. In the slices from rats with prenatal hHCY SD was initiated within 1.4±0.3 min ($p<0.05$; n=11). Also the amplitude SD-like wave increased up to were 0.6±0.7 mV and 7.0±1.5 s (n=1; $p<0.05$). Our results shown that neurons of rat cortex have higher sensitivity to imbalance of the thiols metabolism which may underlie a high risk of appearance migraine headaches with aura in postnatal life in case of maternal hHCY.

This research was funded by RSF№20-15-00100.

DISORDERS OF SYNAPTIC PLASTICITY IN THE YOUNG RAT'S HIPPOCAMPUS FOLLOWING EARLY-LIFE FEBRILE SEIZURES

Griflyuk A.V., Postnikova T.Yu., Amakhin D.V., Soboleva E.B., Zaitsev A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

Griflyuk.Al@mail.ru

Infectious diseases with fever can provoke febrile seizures (FSs), which constitute one of the most common neurological disorders in children between 3 months and five years. As with many other negative factors acting in early life, the FSs can also cause long-lasting alteration of cognitive functions, particularly learning and memory. However, existing data about the impact of FSs on the developing brain are conflicting. We aimed to investigate morphological and functional changes in the hippocampus of young rats exposed to hyperthermia-induced seizures at postnatal day 10. Only animals with prolonged FSs that lasted at least 15 min were included in the experiments. The study was performed 11 days after FSs. We found that FSs led to a slight morphological disturbance. The cell numbers decreased by 10% in the CA1 and hilus but did not reduce in the CA3 or dentate gyrus areas. In contrast, functional impairments were robust. Long-term potentiation (LTP) in CA3-CA1 synapses was strongly reduced in the FS group (1.21±0.06) compared to the control (1.55 ±0.09), which we attribute to the insufficient activity of N-methyl-D-aspartate receptors (NMDARs). We found higher desensitization of NMDAR currents in the FS group using whole-cell recordings. Since the desensitization of NMDARs depends on subunit composition, we analyzed NMDAR current decays, which revealed no differences between control and FS rats. We suggest that increased desensitization is due to insufficient activation of the glycine site of NMDARs, as the application of D-serine, the glycine site agonist, allows the restoration of LTP to a control value. Thus, our results reveal a new molecular mechanism of FS impact on the developing brain.

EB3 PROTEIN POTENTIATE ENDOPLASMIC-RETICULUM LOCALIZATION IN HIPPOCAMPAL DENDRITIC SPINES AND MAKE THEM RESILIENT TO AMYLOID-BETA TOXICITY

Pchitskaya E.[1], Rakovskaya A.[1], Chigray M.[1], Bezprozvanny I.[1,2]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Department of Physiology, UT Southwestern Medical Center at Dallas, Dallas, TX 75390, USA

pchitskaya_ei@spbstu.ru

Dendritic spines are tiny protrusions on neurons that receive most of the excitatory synaptic inputs in the brain. EB3 protein transiently enters the dendritic spines at the tip of the growing microtubule. By manipulating EB3 expression level we have shown, that this protein is important for normal dendritogenesis – EB3 overexpression and knockout reduces hippocampal neurons dendritic branching and total dendritic length. Probably, this effect occurs due to the speeding neuronal development cycle from dendrite outgrowth to the step when dendritic spines are forming. Some of the spines contain endoplasmic reticulum inside it, which is able to form spine apparatus – specialized dendritic spines organelle involved in synaptic plasticity. Implementing direct morphometric characterization of dendritic spines, we showed that EB3 overexpression leads to a dramatic increase in dendritic spines head area, number of ER containing spines and its area inside the spine. EB3 knockout oppositely reduces spine head area and increases spine neck length and spine neck/spine length ratio, alters ER presence in spines. The same effect is observed in conditions of amyloid-beta toxicity, modeling Alzheimer`s disease. Neck elongation is supposed to be a common detrimental effect on the spine's shape, which makes them biochemically and electrically less connected to the dendrite. According to preliminary results EB3 potentates ER movement in dendritic spines through associating with ER resident calcium sensor protein STIM2. EB3 potentiates the formation of presynaptic protein Synapsin clusters and CaMKII-alpha localization in spines rather than in dendrites of hippocampal neurons, while its downregulation has an opposite effect and reduces the size of presynaptic protein clusters Synapsin and PSD95. Such EB3 role in spines development and maturation determines its neuroprotective effect. EB3 overexpression makes dendritic spines resilient to amyloid-beta toxicity, restores altered PSD95 clustering and reduced CaMKII-alpha localization in spines, observed in this pathological state.

Supported by RNF № 21-74-00028.

EFFECTS OF KB-R7943 ON DEPRESSION-LIKE BEHAVIOR IN A RAT MODEL OF STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHIC PAIN

Ivanova N.[1,2], Hristov M.[2], Surcheva S.[2], Shestakova N.N.[1,2,3], Gateva P.[1]

1 - Medical University Sofia, Faculty of Medicine, Sofia, Bulgaria
2 - Institute of Neurobiology, Bulgarian Academy of Sciences (BAS), Sofia 1113, Bulgaria
3 - Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St. Petersburg, Russia

pandreeva_gateva@outlook.com

Diabetes mellitus is one of the most common causes of neuropathic pain, often associated with depression, decreased quality of life, and higher morbidity and mortality. Mood disorders are associated with abnormal neurotransmission. KB-R7943, a Na+/Ca2+ exchanger (NCX) inhibitor, may modify the neurotransmitter release or glutamate receptors activity. The present work aimed to study the effects of KB-R7943 on depression-like behavior in a rat model of streptozotocin-induced diabetic neuropathic pain. Diabetic neuropathic pain was induced by a single intraperitoneal (i.p.) injection of streptozotocin at a dose of 55 mg/kg. After the development of neuropathic pain, KB-R7943 was administered by oral gavage at two amounts: 5 mg/kg and 10 mg/kg for 10 days. Amitryptiline (AMT) was used as a positive control at 10 mg/kg p.o. The following groups were assigned: controls treated with vehicle (C-Veh), rats with neuropathic pain treated with vehicle (NP-veh), rats with neuropathic pain and treated with KB-R7943 at 5 mg/kg (NP-KB-R7943-5mg), rats with neuropathic pain, and KB-R7943 at 10 mg/kg (NP-KB-R7943-10 mg) and rats with neuropathic pain and AMT at 10 mg/kg (NP-AMT-10 mg). The following tests were conducted at the end of the treatment: forced swimming test (FST) - the immobility time (s) (movements to keep the head above the water) for 5 minutes and sucrose splash test (SSPL) - total grooming time for 5 minutes was recorded. Rats with neuropathic pain showed depressive behavior indicated decreased grooming in the SSPL and increased immobility time spent in the FST, which was suppressed by AMT treatment. KB-R7943 of 10 mg/kg reversed the reduced immobility time in the FST of NP-KB-R7943-10 mg group to control level. The present data suggest that chronic KB-R7943 treatment can positively affect the treatment of depression in a rat model of streptozotocin-induced diabetic neuropathic pain.

Acknowledgments: This work was supported by RFBR grant 20-515-18008, the National Science Fund of Bulgaria Grant КП-06-Rusia 25/16.12.2020

EARLY DYSFUNCTION OF GLIAL CELLS AS A POSSIBLE PREMISE OF ALZHEIMER'S DISEASE

Rudnitskaya E.A., Kozlova T.A., Burnyasheva A.O., Stefanova N.A., Kolosova N.G.

Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

ekaterina.rudnitskaia@gmail.com

Recent studies using state of the art diagnostic techniques have confirmed that the preclinical period of sporadic (> 95% of cases) form of Alzheimer's disease (AD) can last for decades, but the question of when exactly the disease development begins and what contributes to its development remains open. Several epidemiological and experimental studies indicated that the predisposition for accelerated aging, the main risk factor for AD development, can be formed during the early postnatal period of life, at the age of the completion of brain maturation. The results of our work on the senescence-accelerated OXYS rats, a unique model of sporadic AD, have confirmed the validity of this hypothesis. We have identified the features of brain maturation in OXYS rats in the early postnatal period (at the ages P0-P20, P – postnatal day) which can act as prerequisites for the development of initial neurodegenerative changes in the later age. OXYS rats have a lower (comparing with their maternal Wistar strain) body weight at birth, decreased duration of gestation, a delayed physical development and formation of reflexes in the postnatal period against the background of impaired neuronal stem cells differentiation and the formation of mossy fibers in the dentate gyrus of the hippocampus, and a decrease in the efficiency of formation of synaptic contacts in OXYS rats. The completion of brain maturation in OXYS rats occurs against the background of a decrease in astrocytic and microglial support – a key regulator of the neural circuitry function – taking place in the hippocampus and prefrontal cortex. The lack of glial support may be the cause of the decreased efficiency of the formation of synaptic contacts found in OXYS rats, which made it possible to consider it as a key event leading to the development of AD signs in the future.

Supported by RSF (grant #19-15-00044)

EARLY PERIOD OF TRAUMATIC BRAIN INJURY IN RATS ASSOCIATED WITH SPATIAL BUT NOT OBJECT MEMORY DISTURBANCES IN RATS

Shalneva D.V.[1], Komoltsev I.G.[1,2], Frankevich S.O.[1,2], Volkova A.A.[1], Shirobokova N.I.[1], Salyp O.Y.[1], Bashkatova D.A.[1], Kostyunina O.V.[1], Chizhova O.A.[1], Kostrukov P.A.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Department of Functional Biochemistry of Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

dariashal1999@gmail.com

Introduction: Most patients after traumatic brain injury (TBI) suffer from long-term complications, including memory disturbances. Previously we demonstrated early bilateral hippocampal damage in rats after TBI. We hypothesized that hippocampal damage is possibly associated with early hippocampal-dependent spatial working memory dysfunction in rats.

Methods: The experiment was performed on 51 male Sprague-Dailey rats, divided into TBI, Sham, and control groups. Memory disturbances were assessed using the object location test (OLT) and new object recognition test (NORT) to verify spatial and object memory, respectively. Testing included 4 sessions. The habituation session was performed on day 4 after TBI, rats freely investigating the arena for 10 minutes. On day 5 we put into the arena two same objects and let rats investigate it for 5 minutes (training session). Then, two sessions 30 min apart were performed: first, one of the objects was moved to a new corner of the arena (the OLT session). Second, the other object was replaced with a novel object (the NORT session). Data was analyzed using Noldus EthoVision XT.

Results: During the habituation session rats after TBI investigated the objects for less time compared with rats of the Sham group. During the OLT session, rats after TBI were sniffing non-replaced object for 9±2s, and replaced object for 5±1s ($p<0.05$). On the contrary, the sham group preferred the replaced object (29±7s) to the non-replaced one (12±5s, $p<0.05$). During the NORT session, there were no significant differences in the time of investigating objects between groups.

Conclusion: The data demonstrated location but not object memory disturbances in the early posttraumatic period in rats, representing specific spatial working memory decline. Behavioral abnormalities may reflect early hippocampal dysfunction after TBI.

Disclosures. Supported by RSF, grant №22-25-00713

EFFECTS OF DIMETHYL SULFOXIDE ON HIPPOCAMPAL NEURAL ACTIVITY

Darbinyan L., Simonyan K., Hambardzumyan L., Manukyan L., Sarkisian V.

Orbeli Institute of Physiology, Yerevan, Armenia

livada0106@gmail.com

Dimethyl sulfoxide (DMSO) is widely used in preclinical and clinical research, as it enhances the entrance of water-insoluble drug candidates into the central nervous system. DMSO suppresses the inward and outward currents by interacting with GABA receptor-Cl- channel complex. It was shown that, particularly low DMSO concentrations appear to profoundly influence neural network activities. Modulation of cell signaling by low concentrations of DMSO could also explain the differential regulation of neuronal activation in various brain regions. There is no evidence for accumulation of DMSO in the brain tissue and the elimination occurs within 12–36 h in experimental animals. Background and evoked spike activity were recorded in single neurons of the hippocampus treated with DMSO (1 ml/kg /kg, i.p., once) (n=5) in the dynamics (from 1 to 105 minutes) after DMSO exposure (after 5 minutes). The main effects lasted up to 40 and 90 minutes. In response to HFS (High frequency stimulation) of the Entorhinal cortex, the analysis revealed inhibitory effects.

EFFECTS OF AURANOFIN TREATMENT ON BRAIN PROTEOME DURING ISCHEMIC STROKE

Tereshchuk V.M.[1,2], Nesterenko A.M.[1,3], Bilan D.S.[1,2], Kelmanson I.V.[1,2], Kostyuk A.I.[1], Panova A.S.[1], Kotova D.A.[1], Kokova A.D.[1], Graf I.[4], Doeppner T.R.[4], Belousov V.V.[1,2,3]

1 - Institute of Bioorganic Chemistry of Russian Academy of Sciences, Moscow, Russia
2 - Pirogov Russian National Research Medical University, Moscow, Russia
3 - Federal Center of Brain Research and Neurotechnologies, Federal Medical Biological Agency, Mosco, Russia
4 - Department of Neurology, University Medical Center Göttingen, Göttingen, Germany

vasmter@gmail.com

Auranofin is a drug which disrupts thioredoxin antioxidant system. In turn, disruption of thioredoxin system causes changes in intracellular redox balance. Due to the fact that changes in redox balance take place after ischemia, the aim of the study was to estimate effects of auranofin treatment and following redox changes on ischemic brain. Using MCAO in mice there was developed a model for proteomics analysis of ischemic mouse brains with or without auranofin treatment.

Proteomic analysis of ischemic brain tissue using LC-MS/MS allowed us to detect changes

on protein level and further to conduct differential enrichment analysis of proteomics data. The analysis revealed a number of genes whose expression was significantly altered during stroke. Based on the obtained data on differential expression, a set of genes was selected for further analysis. There was also used a comparison dataset of ischemic mouse brain tissue from five time points up to 28 days. First, LDA was performed on a comparison dataset, then auranofin-based data was projected on these coordinates. The result of this projection showed that data corresponding to auranofin-treated brains is located closer to the area of the non-ischemic or recovering hemispheres. This suggests that auranofin treatment shows recovery effects at the proteomic level.

Supported by RSF (project No. 21-74-00140)

EFFECTS OF MEMBRANE CHOLESTEROL OXIDATION ON SYNAPTIC VESICLE CYCLING AT THE MICE NEUROMUSCULAR JUNCTIONS

Zakirianova G.F.[1,2], Giniatullin A.R.[1], Gafurova C.R.[1,2], Petrov A.M.[1,2]

1 - Institute of Neuroscience, Kazan State Medical University, Kazan, Russia
2 - Laboratory of Biophysics of Synaptic Processes, Kazan Institute of Biochemistry and Biophysics, Federal Research Center "Kazan Scientific Center of RAS", Kazan, Russia

farraguz12@gmail.com

Neuromuscular transmission is the main process underling translation of commands from motor neurons to the skeletal muscle. A reason of neuromuscular defects may be disruptions in metabolism of cholesterol, whose content is high in the motor nerve terminals. Using electrophysiological and fluorescent approaches the effects of cholesterol oxidase (ChO) at high and low concentrations (0.2 and 2 U/ml; 20 min application) were studied in the mice diaphragm.

It has been found that ChO effectively oxidized membrane cholesterol and caused defects in synaptic membranes, namely, lipid raft disruption and increase in membrane fluidity. At high concentration, ChO also affected extrasynaptic membranes of the muscle fibers. Oxidation of membrane cholesterol had no influence on neurosecretion in response to low frequency (0.5 Hz) activity, but markedly increased neurotransmitter release at both moderate (20 Hz) and high (70 Hz) frequency stimulation. Evaluation of synaptic vesicle (SV) exo-endocytosis cycling with FM dyes and sulforhodamine 101 (FM1-43 quencher able to pass through fusion pore) showed that the cholesterol oxidation enhanced exo-endocytosis of SVs at both 20 and 70 Hz. In addition, ChO pretreatment stimulated switching of SV exocytosis from “kiss-and-run” to full-collapse fusion mode in response to high frequency activity. To oxidize cholesterol of SVs, the exocytosis and compensatory endocytosis were induced during application of ChO. In this case, the enzyme gains access to the SV membranes incorporated into the presynaptic membrane after exocytosis, but

before endocytosis. Under these conditions, cholesterol oxidation markedly depressed neurotransmitter release at moderate and high frequencies of activity due to suppression of SV mobilization to exocytosis sites.

Thus, oxidation of cholesterol from plasma membrane and SVs can affect SV mobilization during intense activity in opposite directions. This can reflect different roles of lipid rafts in presynaptic and SV membranes.

The study was supported by Russian Science Foundation (grant #21-14-00044).

ENDOGENOUS CATECHOLAMINES AND EXOGENOUS SYMPATHOMIMETICS DIFFERENTLY MODULATE THE SYNAPTIC TRANSMISSION IN MUSCLES OF VARIOUS PHYSIOLOGICAL PROFILE

Bukharaeva E., Tsentsevitsky A., Khuzakhmetova V., Arkhipov A., Zilyakov N., Sibgatullina G.

Kazan Institute of Biochemistry and Biophysics Kazan Research Center "Kazan Scientific Centre RAS", Kazan, Russia

ellyab@mail.ru

We have analyzed the adrenaline, noradrenaline, and some sympathomimetics action on the acetylcholine (ACh) quanta secretion at the neuromuscular junctions of the muscles of different functional profiles. Using enzyme immunoassay, it was found that the solution washing the neuromuscular preparation of the mouse diaphragm and locomotor muscles contains up to 12 pg/ml of adrenaline and 62 pg/ml of norepinephrine. Thus, we confirmed the microscopy data that sympathetic nerve endings near the synapse release endogenous catecholamines and can be able to modulate synaptic transmission. Significant differences were found in the direction and severity of the effects of adrenaline, norepinephrine, and specific agonists and antagonists of different types of adrenoreceptors on the spontaneous and evoked secretion of ACh quanta in synapses of muscle different functional profiles. In the synapses of the diaphragm, activation of the α1, α2, and β1 receptor subtypes inhibited the spontaneous secretion of ACh, while, on the contrary, its increase was observed in the synapses of the locomotor muscles. Upon stimulation of the motor nerve in the synapses of the diaphragm, activation of α2 and β1 subtypes caused inhibition of ACh secretion, and activation of β2 caused its increase. In the synapses of the locomotor muscle of the "fast" type, adrenaline increased the number of quanta released in response to a nerve stimulus, norepinephrine did not act, and in the "slow" type muscle, adrenaline increased the quantal content. The observed differences in the effects of adrenergic drugs on ACh secretion are due to their interaction with different subtypes of adrenoreceptors that activate various intracellular molecular cascades. It is important to take into account the peculiarities of the action of catecholamines in muscle synapses

of different functional profiles when developing new drugs for the treatment of diseases with synaptic defects. Supported by the Russian Science Foundation (18-15-00046) and government assignment for FRC Kazan Scientific Center of RAS.

ETHANOL WEAKENS TRICYCLIC ANTIDEPRESSANT ACTION ON NMDA RECEPTORS OF CORTICAL NEURONS

Boikov S.I., Sibarov D.A., Antonov S.M.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St.Petersburg, Russia

sergei-boickov@mail.ru

Tricyclic antidepressants (TCAs) are known to block NMDA receptor (NMDAR) currents. We have also previously demonstrated that at therapeutically relevant concentrations amitriptyline and desipramine act on NMDARs in a calcium-dependent manner. Ethanol is also known to produce calcium-dependent inhibition of NMDARs. Here we study the effect of TCAs in control and in the presence of 0.03% ethanol, which corresponds to mild intoxication when measured in blood plasma. The effects of three TCAs (amitriptyline, desipramine, clomipramine) on NMDA-mediated currents were investigated using patch-clamp method in the "whole cell" configuration on neurons in the primary culture from rat neocortex. In the presence of 2 mM extracellular calcium, TCAs inhibited NMDA elicited currents with IC_{50}s of 5 μM for amitriptyline and 1.7 μM for desipramine. Ethanol addition increased amitriptyline and desipramine IC_{50}s to 132 μM and 11 μM respectively. These IC_{50}s coincide to "pure" open-channel block of NMDARs by these TCAs obtained in calcium-free solution. Therefore the presence of ethanol probably eliminates the calcium-dependent inhibition of NMDARs by amitriptyline and desipramine, but do not affect calcium-independent channel-block at high TCA concentrations. Inhibition of NMDAR currents by clomipramine at physiologically relevant extracellular calcium concentrations did not depend on the presence of ethanol. Thus ethanol abolished both amitriptyline and desipramine produced calcium-dependent inhibition of NMDARs. Conversely, clomipramine effects did not depend on extracellular calcium and ethanol. It is likely that ethonol may considerably complicate the TCAs usage as drugs and interfere with the amitriptyline and desipramine therapeutic effects.

The study was supported by Russian Science Foundation grant 21-15-00403.

EVIDENCE OF THE INVOLVEMENT OF CGRP-RECEPTORS IN 2-AG-INDUCED POTENTIATION OF TRANSMITTER RELEASE IN MOUSE MOTOR SYNAPSES

Tarasova E.O., Khotkina N.A., Gaydukov A.E., Balezina O.P.

Lomonosov Moscow State University, Moscow, Russia

cate1990@list.ru

2-arachidonoyl-glycerol (2-AG) is a widely studied endocannabinoid. In the central nervous system this substance acts as retrograde regulator which generally suppresses transmitter release by acting on CB-receptors of the presynaptic terminal. In peripheral neuromuscular junctions (NMJs) we were the first to show that exogenous application of 2-AG leads to an increase in the amplitude of miniature end plate potentials (MEPPs) through CB1-receptor activation and thereby potentiates synaptic transmission. The following work was focused on elucidating the mechanisms of such a 2-AG-mediated potentiation of the activity of NMJs.

Experiments were conducted on isolated neuromuscular preparations of mice semi-diaphragms with use of the standard microelectrode technique of intracellular biopotential registration. Obtained data was further analyzed in MiniAnalysis (Synaptosoft, United States) and GraphPad Prism 6.0.

The increase in MEPP amplitude caused by 2-AG (1 μM) was completely prevented by vesamicol (1 μM) which is a blocker of the vesicular acetylcholine transporter. Thus, 2-AG potentiates synaptic transmission by increasing the size of the quantum of acetylcholine (ACh). PKA inhibition by H-89 (1 μM) also prevented the 2-AG mediated increase in MEPP amplitude, indicating that PKA is a part of a signaling pathway which is triggered by 2-AG action and leads to an upregulation of ACh pumping into vesicles. We previously observed such PKA-dependent increase in the size of ACh quanta upon CGRP-receptor activation. This made us wonder whether CGRP-receptors are also involved in the effects of 2-AG. Blocking CGRP-receptors with CGRP8-37 completely averted the effects of 2-AG on MEPP amplitude. Thus, we are the first to show an involvement of CGRP-receptors in the 2-AG-induced potentiation of ACh release in mouse NMJs. The details of the intertwining of CGRP-mediated and 2-AG-mediated signaling pathways in mouse motor synapses is the subject of further research.

EXPRESSION OF γ-CRYSTALLIN GENES IN THE LENSES OF TWO FISH SPECIES

Kapitunova A.I., Dominova I.N., Zhukov V.V.

Immanuel Kant Baltic Federal University, Kaliningrad, Russia

irinadominova@gmail.com

γ-Crystallins occupy a quantitatively dominant position among fish lens proteins. Most of the proteins in this group are γM-crystallins. The present study is aimed to identification of some γ-crystallin genes in the lens of the juvenile specimens *Cyprinus carpio* and *Sander lucioperca*, and the calculation of the concentration increment of the refractive index (dn/dc) of these crystallins.

For this purpose, we extracted RNA from the lenses and carried out qPCR. The transcription levels (TLs) γ-crystallin genes were normalized to the 18S rRNA gene and calculated using the 2ddCt method. Statistical analysis was performed using the t-test ($p < 0.05$). The crystallin dn/dc values were measured as weighted average meanings based on protein amino acid composition and individual amino acid dn/dc.

So, we determined 7 transcribed γ-crystallin genes in the lens of the carp: *Crygn1*, *Crygs2*, *Gcm1*, *Gcm1l*, *Gcm2l, Gcm2l2*, *Crygm6*, and 6 genes in the lens of the pikeperch: *Gcm2l*, *Gcm2l2*, *Gcm2l3*, *Gcm2l4*, *Gcm2l5*, *Crygs*. Analysis of the gene TLs expressed in the lens of carp and pikeperch revealed the downregulation of *Gcm2l*, *Gcm2l2*, *Crygm6*, *Crygn* in pikeperch compared to carp genes.

Calculation of the γ-crystallins dn/dc demonstrated the higher values for carp proteins compared to pikeperch but the differences were only in thousandths. Furthermore, the higher dn/dc was obtained for γM-crystallins of both fish species. However, the average dn/dc values exceed ones of the other crystallins by values within hundredths.

It is assumed that the high refractive index value of the fish lens is due to the γM-crystallins, which contain a large proportion of methionine. However, methionine itself shows no greater value either in terms of the molar refraction magnitude or in the dn/dc value. Probably, to obtain high refractive index values, protein folding plays a more significant role compared to their amino acid composition.

FABP-7 DEVELOPMENTAL IMMUNOMORPHOLOGY OF TELENCEPHALON AT THE EARLY HUMAN FETUSES: PRELIMINARY RESULTS

Kharlamova A.S.[1], Tsvetkova E.G.[1], Godovalova O.S.[2], Junemann O.A.[1], Proschina A.E.[1]

1 - A.P. Avtsyn Research Institute of Human Morphology, Moscow, Russia
2 - GBUZ MONIIAG, Moscow, Russia

grossulyar@gmail.com

A brain-fatty acid-binding protein (FABP-7) – one of the three FABPs involved in the brain early development – was hypothesized to participate in radial glia establishment and neuroblast migration. It was supposed to involve in Down syndrome and schizophrenia, participate in astrocyte proliferation, but not migration, during CNS injury. This study is a part of the human brain development project: more than fifteen fetal brain samples aged from eight pcw to birth had been studied with a wide panel of markers, including FABP-7 antibody.

At the prefetal period (8pcw-13gw) FABP-7-immunorecrive neuroblasts appeared in restricted zones of dorsolateral lateral ganglionic eminence (LGE) surface area and short field in the adjunct intermediate neocortical zone, olfactory bulb and septal ventricular zones. No immunoreactive cells were revealed within ventricular and subventricular zones. Separate FABP-7-neuroblsts were observed in striatum. FABP-7-immunoreactive fibers, not neuroblasts, were located in ventral intermediate neocortical zone, lateral migratory curve, insular cortex, internal capsule. At 14gw FABP-7-immunoreactivity was demonstrated throw the whole intermediate zone of neocortex. FABP-7-neuroblasts also appeared in deep medial ganglionic eminence, separate neuroblasts – in caudate nucleus and putamen, paleocortical plate, diagonal band and substantia innominata. At the 15,5gw and later FABP-7-neuroblasts were revealed in deep LGE, but not in the dorsal surface, amygdala, and were abundant in striatum from this stage. This immunoreactivity pattern continued up to the end of early fetal period (20-21gw). Only at the middle fetal period FABP-7-neuroblasts were described in neocortical, insular and entorhinal cortical plates, while in ganglionic eminence FABP-7-immunoreactive cells were demonstrated only in the deep bandelet bordered with dorsal caudate nucleus. Thus, FABP-7 immunoreactivity distribution homology at the developmental telencephalon between human and modal animals becomes unclear: no FABP-7-immunoreactive cells were observed at the ventricula/subventricular zones of neocortex in early fetal development.

This study is supported by the Russian Science Foundation (RSF) grant #22-25-00370

FEATURES OF THE BLOOD-BRAIN BARRIER FORMATION IN SENESCENCE-ACCELERATED OXYS RATS DURING EARLY POSTNATAL DEVELOPMENT

Kozlova T.A., Tyumentsev M.A., Rudnitskaya E.A., Stefanova N.A.

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

kozlovatanya21@gmail.com

Alzheimer's disease (AD) is detrimental neurodegenerative disorder which development is accompanied by dysfunction of the blood-brain barrier (BBB) and decrease of the cerebral blood flow. Prerequisites of these alterations may originate at early age during BBB formation; however a possible interconnection between altered BBB formation and its dysfunction later in life is poorly understood. For this purpose suitable animal models of AD are needed. Senescence-accelerated OXYS rats are considered as an adequate model of the most common sporadic form of AD because of development of all key signs of the disease without mutations in *App*, *Psen1* and *Psen2* genes.

Here we investigate the formation of BBB in the hippocampus and frontal cortex of OXYS and Wistar (control) rats during early postnatal development. We studied the number and length of tight junctions (TJ) between endothelial cells as well as the distance between TJ using electron microscopy and levels of occludin and claudin 5 as key TJ proteins by Western blot analysis. Besides, we estimated the size and shape of capillary lumensas well as the integrity of a basement membrane.We found that the level of occludin naturally increased from birth to the end of the second postnatal week (postnatal day 14) in rats of both strains. At the same time, at postnatal day 10 the level of occludin was higher in the frontal cortex of OXYS rats compared to Wistar rats. Besides we found that ultrastructure of capillaries was altered in the hippocampus of OXYS rats at early postnatal age.

Alterations of the BBB formation may result in cerebrovascular dysfunction observed previously in OXYS rats even at the early stage of AD-like pathology which in turn may contribute to the progression of neurodegeneration late in life.

This work was supported by Russian Science Foundation (grant #19-15-00044).

GABA-ERGIC NEUROTRANSMISSION IN THE HIPPOCAMPUS OF KRUSHINSKY-MOLODKINA RATS IN THE FORMATION OF AUDIOGENIC EPILEPSY DURING POSTNATAL ONTOGENESIS

Ivlev A.P., Aleksandrova E.P., Nikolaeva S.D., Chernigovskaya E.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

andrewivlev1410@gmail.com

The hippocampus plays an important role in audiogenic reflex epilepsy progression. Earlier it was shown, that maturation of the hippocampus of Krushinsky-Molodkina (KM) rats, which is a genetic model of heritable audiogenic reflex epilepsy, takes about 2 months, which is longer as compared to Wistar rats (Kulikov *et al*, 2020), but there was no data on development of GABA-ergic system. That is why the aim of study was to assess the features of the development of GABA-ergic neurotransmission in the hippocampus of KM rats in the formation of audiogenic epilepsy during postnatal ontogenesis.

The study was carried out on naive KM rats at age of 15, 60 and 120 postnatal days (P15, P60 and P120 respectively). Wistar rats of the corresponding age were used as control. After dissection, the hippocampi of rats were analyzed by Western blotting, immunohistochemistry and PCR-RT methods.

At P15 we found a decrease in the amount of GAD67 and parvalbumin, proteins involved in GABA transmission, and an increase in levels of VGAT and $GABA_A$ receptor in the hippocampus of KM rats in comparison with control. At P60 there was an increase in the content of GAD67 and parvalbumin and a decrease in levels of VGAT and $GABA_A$ receptor, which is also observed in the hippocampus of adult rats with formed convulsive readiness. Chlorine homeostasis, the main factor in the work of GABA-ergic neurotransmission, undergoes changes during ontogenesis and in neurodegenerative diseases. At all time-points a decreased activity of the chlorine exporter KCC2 in the hippocampus of KM rats is observed, which may indicate the excitatory activity of $GABA_A$ receptors. Thus, later maturation of the hippocampus is one of the reasons for the disruption of the GABA-ergic system in KM rats during ontogenesis, which may be a precondition for the development of convulsive readiness.

GENERATION OF NEURONAL CELLS FROM SKIN FIBROBLASTS BY TRANSDIFFERENTIATION TO STUDY HUNTINGTON'S DISEASE PATHOLOGY

Kraskovskaya N.A.[1,2], Bezprozvanny I.B.[2,3]

1 - Center for Cellular Technologies, Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russia
2 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
3 - Department of Physiology, UT Southwestern Medical Center, Dallas, Texas

ninakraskovskaya@gmail.com

Many pathological features of neurodegenerative diseases, such as the development of synaptic dysfunction, appear with age. Trans differentiation or direct reprogramming allows you to save the age of obtaining neurons. However, the low efficiency of direct reprogramming methods limits their application in research practice.

In the present study, we improved an existing direct reprogramming protocol based on the use of microRNAs, transcription factors, and small molecules, which allowed us to obtain a population of neuronal cells from dermal fibroblasts with an efficiency of 80 percent. We have obtained a homogeneous population of induced medium spiny neurons (iMSN), the most vulnerable cell type in Huntington's disease (HD). MSN degeneration leads to the development of motor disorders in patients. Induced excitatory neurons (iEN) can also be obtained from primary fibroblasts to study the pathogenesis of cognitive impairments observed in the case of HD. Both iMSN and iEN are positively stained for specific neuronal markers and respond to potassium chloride and glutamate stimulation. In addition, reprogrammed neurons have the inherent electrophysiological activity of this cell type, and iEN is capable of generating spontaneous activity. Moreover, reprogrammed neurons are capable to form synaptic connections which makes it possible to study defects in synaptic transmission during the development of neurodegenerative changes in cells.

Thus the modified protocol makes it possible to obtain a homogeneous population of iEN and iMSN with high efficiency, which significantly reduces the variability in morpho-functional studies and facilitates the interpretation of the results. This protocol might be useful in both studying the molecular and cellular basis of HD pathogenesis and drug discovery as well as in the development of a personalized approach for therapy.

HUMAN NEUROMODULATOR LYNX2: STRUCTURE, TARGET AND IMPACT ON COGNITIVE-RELATED PROCESSES

Lyukmanova E.[1,2], Bychkov M.[1], Kulbatskii D.[1], Zaigraev M.[1,3], Kocharovskaya M.[1,3], Shulepko M.[1], Chugunov A.[1], Kirpichnikov M.[1,2], Shenkarev Z.[1], Paramonov A.[1]

1 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia
2 - Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology», Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia
3 - Moscow Institute of Physics and Technology (State University), Dolgoprudny, Moscow Region, Russia

ekaterina-lyukmanova@yandex.ru

Human regulatory proteins from the Ly6/uPAR family share structural homology with snake α-neurotoxins and modulate functions of nicotinic acetylcholine receptors (nAChRs). Some of them (Lynx1, Lypd6) are localized predominantly in the nervous system and were shown to regulate cognitive processes in the brain. Lynx2 is one of the poorly studied members of the Ly6/uPAR family. It was known that Lynx2 is tethered to the cell membrane by GPI anchor, binds to α4β2-nAChRs and is involved in the anxiety-related behavior.

In our work we used recombinant water-soluble variant of human Lynx2 without GPI-anchor. We found that Lynx2 extracts from the brain homogenate α4-, α6-, and β2- nAChR subunits and α5- and γ2-subunits of the $GABA_A$ receptor. Electrophysiology study of Lynx2 in *Xenopus* oocytes revealed selective inhibition of "low sensitive" α4β2-nAChRs having 3xα4+2xβ2 architecture. This allowed to hypothesize that the Lynx2 binding site is located at the α4/α4 interface of the receptor. Using NMR, we determined the Lynx2 structure, that in turn allowed us to study the Lynx2 interaction with α4β2-nAChR *in silico*. Lynx2 binding with the α4/α4 interface of the receptor was confirmed and amino acid residues important for this interaction were revealed.

Expression of genes coding different synaptic plasticity markers and miRNAs in the neuronal culture treated by recombinant Lynx2 was analyzed. Lynx2 down-regulates transcription of the receptors and messengers implicated in long-term potentiation (LTP) and increases expression of miR-451, which inhibits the neuronal differentiation. In line with these data, Lynx2 inhibited LTP in mouse hippocampal slices and reduced the number of stub-shaped and mushroom-shaped dendritic spines in the cultured neurons.

Thus, Lynx2 plays the important regulatory role in the brain processes related with cognitive function.

Work is supported by the Russian Science Foundation (Project 19-74-20176).

HINDLIMB UNLOADING INDUCES AUTOPHAGY AND APOPTOSIS IN THE HIPPOCAMPUS OF RATS

Oleinik E.A.[1], Grigoreva Y.S.[1], Tyganov S.A.[2], Glazova M.V.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia
2 - Institute of Biomedical Problems, Russian Academy of Sciences, Moscow, Russia

ekaterina.oleynik96@mail.ru

Microgravity is known to cause various neurochemical changes in a brain. Previously we demonstrated that short-term hindlimb unloading (HU) leads to activation of Akt-dependent neuroprotective mechanism in the hippocampus. On the other hand, the autophagy and apoptosis are the key mechanisms for maintaining homeostasis and cell survival. Thus, here we analyzed the autophagy and apoptosis in hippocampus at different time points of HU.

Adult male Wistar rats were divided into 5 groups: isolated control (C); 1-day HU; 3HU; 7HU and 14HU. Our results showed that after 3-day HU the expression of autophagy markers, p62 and LC3B, was significantly decreased, which suggested an activation of autophagy. Immunofluorescence data also demonstrated the assembly of autolysosomes and the activation of autophagy in the hilus and CA4 of the hippocampus in 1HU and 3HU rats. However, after long-term HU (7HU, 14HU), no differences in the expression of the autophagy proteins were detected any longer.

In addition, we observed the activation of caspase 8 after 3HU, which may indicate of apoptosis induction. At the same time, we did not show significant changes in the number of cells in the dentate gyrus, hilus and CA3. However, after 7HU, we observed a significant decrease in the expression of the Bcl-2 and in increase of p53 phosphorylation, which could indicate the presence of cellular stress and triggering of apoptotic cell death. Indeed, in the hippocampus of 7HU rats, there was a decrease in the number of cells in the granular layer of the dentate gyrus.

Thus, we demonstrated the activation of autophagy after short-term HU (1HU, 3HU), which indicates the activation of the survival processes of hippocampal neurons. In opposite, prolonged hindlimb unloading led to the activation of apoptosis and cell death in the dentate gyrus.

Supported by RFBR, grant number 20-015-00062.

HIPPOCAMPAL GLUTAMATERGIC SYSTEM OF THE KRUSHINSKY-MOLODKINA RATS DURING THE DEVELOPMENT OF TEMPORAL LOBE EPILEPSY

Aleksandrova E.P., Kulikov A.A., Ivlev A.P., Chernigovskaya E.V.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

alyx-katich@mail.ru

Krushinsky-Molodkina (KM) rats are genetically prone to audiogenic seizure and used as a model of reflex epilepsy. However, as the result of repeated stimulation, epileptic activity occupies the limbic system of the KM rat's brain. This process is called audiogenic kindling and models temporal lobe epilepsy.

We studied the chronic changes in molecular mechanisms, regulating hippocampal glutamatergic neurons of KM rats, caused by audiogenic kindling of different durations.

KM rats were stimulated once or daily for 14 or 21 days. Material was retrieved in a day or after a week of the latest stimulation and was analyzed by immunohistochemical staining or by Western-blotting.

Transcription factors CREB and c-Fos activate in glutamatergic neurons after the first stimulation, indicating early involvement of the limbic system in seizure activity. Both in the early stage (14 days of stimulation) and late stage (21 days of stimulation) of the temporal lobe epilepsy development activity of ERK1/2 kinases as well as the expression of ERK-dependent factors CREB, c-Fos and FRA-1 in the glutamatergic neurons remain high.

A day after the last of stimulations, transport and exocytosis of glutamate in hippocampus decreases, as evidenced by the reduced expression of VGLIT1/2, SNAP25, SV2 and the decreased activity of synapsin-1.There is also a decline in content of glutamatergic receptors NMDA and AMPA. However, after a week of rest, the amounts of VGLUT1/2 and glutaminase increase, while levels of active ERK, FRA-1 and p-CREB remain high. This indicates chronic disorders in the functioning of glutamatergic neurons in the hippocampus, that are also confirmed by increased amounts of synaptopodin protein, a marker of the synaptic dendritic apparatus. Increased synaptopodin in areas where the glutamatergic cells dendrites are localized indicates a growth in the number of dendritic contacts and a reorganization of neuronal connections in the hippocampus during the development of temporal lobe epilepsy.

HISTOLOGICAL STAINING OF UNUSUAL CELLS IN SPINAL NERVE ROOTS

Sobol C.V., Gapanovich S.O., Abushik P.A., Vasiliev D.S.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

peep9@yandex.ru

In our previous study we found that frog spinal nerve roots, being loaded by Fura 2 AM, had unusual cells, which are not typical for spinal cord roots. These cells were responded to glutamate and looked like neurons. Therefore, in this study, we tried to assess cell morphology in VIII, IX, and X roots of the frog spinal cord using morphological and immunocytochemical methods.

In one case, after visualization of the cells stained by Fura 2 AM in X root, a histological examination was carried out. Along with cells with typical morphological features of glial cells, more large cells (about 5-9 mkm in size) with spindle-shaped or rounded bodies with outgoing processes were found. The bodies of such cells were located in small groups (3-7 cells each). The space between the bodies of these cells was filled with nerve fibers and the bodies of typical small glial cells.

In the next experiment, the spinal cord with roots was exposed to primary antibodies anti-tubulin III (1:1000, Abcam, ab7751, England) without initial visualization of the cells stained by Fura 2 AM. As a result, we found only one cell in IX ventral root. III-tubulin is a class of neuron-specific tubulin used as one of the mature neuron markers.

Thus, histological methods additionally support our preliminary idea that roots of spinal cords may contain cells with probable neuronal origin. The role and function of these cells found in the spinal cord roots remain unclear.

This work was supported by a state contract with the Federal Agency for Scientific Organizations of Russia (project no. AAA-A18-118012290371-3)

HUMAN NEUROMODULATOR LYPD6 INHIBITS CHOLINERGIC SYSTEM IN THE BRAIN

Kulbatskii D.S.[1], Shenkarev Z.O.[1], Bychkov M.L.[1], Shulepko M.A.[1], Povarov I.S.[2], Popov A.V.[1,3], Peigneur S.[4], Chugunov A.O.[1,5], Sharonova I.N.[2], Skrebitsky V.G.[2], Tytgat J.[4], Kirpichnikov M.P.[1,6], Lyukmanova E.N.[1,6]

1 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences (RAS), Moscow, Russia
2 - Brain Research Department, ResearchCenter of Neurology, Moscow, Russia
3 - Institute of Neuroscience, Lobachevsky Nizhny Novgorod State University, Nizhny Novgorod, Russia
4 - Toxicology and Pharmacology, University of Leuven (KU Leuven), Leuven, Belgium
5 - International Laboratory for Supercomputer Atomistic Modelling and Multi-Scale Analysis, National Research University Higher School of Economics, Moscow, Russia
6 - Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology», Biological Faculty, Lomonosov Moscow State University, Moscow, Russia

d.kulbatskiy@gmail.com

Lypd6 is a GPI-tethered protein from the Ly-6/uPAR family, which was shown to take part in regulation of both cholinergic and Wnt/β-cathenin signaling and which is expressed in mammalian central nervous system. Cholinergic signaling in CNS is involved in such important functions as learning and memory, as well as modulation of neuronal plasticity in postnatal development. Some of the neuropathological conditions observed in Alzheimer' disease, Parkinson disease, epilepsy, and schizophrenia are associated with dysfunction of cholinergic signaling. The endogenous regulatory proteins of cholinergic systems present a great interest as prototypes for new drugs for treatment of cognitive dysfunctions. That's why we focused our study on Lypd6.

To investigate a cholinergic activity of Lypd6, we studied a recombinant water-soluble variant of the human protein (ws-Lypd6) containing isolated "three-finger" LU-domain without the GPI-anchor. The negative allosteric modulatory activity of ws-Lypd6 was demonstrated at a3b4- and a7-nAChRs in *X. laevis* oocytes, and ws-Lypd6 did not elicit currents through nAChRs in absence of ACh. Application of ws-Lypd6 significantly inhibited choline-evoked currents at a7-nAChRs in rat hippocampal slices. Similar to snake neurotoxin a-bungarotoxin, ws-Lypd6 suppressed the long-term potentiation (LTP) in mouse hippocampal slices. Colocalization of endogenous GPI-tethered Lypd6 with a3b4- and a7-nAChRs was detected in primary cortical and hippocampal neurons. Ws-Lypd6 interaction with the extracellular domain of a7-nAChR was modeled using the ensemble protein-protein docking protocol. The interaction of all three Lypd6 loops ("fingers") with the entrance to the orthosteric ligand-binding site and the loop C of the

primary receptor subunit was predicted. The results obtained allow us to consider Lypd6 as the endogenous negative modulator involved in the regulation of the cholinergic system in the brain.

This work was supported by RFBR (project# 20-04-00830).

INTRACELLULAR ELECTROCHEMICAL STUDY OF BETA AMYLOID-INDUCED OXIDATIVE STRESS

Vaneev A.N.[1,2], Timoshenko R.V.[1], Klyachko N.L.[2], Korchev Y.E.[1,3], Gorelkin P.V.[1], Erofeev A.S.[1,2]

1 - National University of Science and Technology "MISIS", Moscow, Russia
2 - Lomonosov Moscow State University, Moscow, Russia
3 - Imperial College London, London, UK

vaneev.aleksandr@gmail.com

Oxidative stress is known to play an important role in the pathogenesis of neurodegenerative diseases. Histopathological hallmarks of AD are intracellular neurofibrillary tangles and extracellular formation of senile plaques composed of the beta-amyloid peptide in aggregated form along with metal-ions such as copper, iron or zinc. Redox active metal ions, as for example copper, can catalyze the production of reactive oxygen species (ROS) when bound to the beta-amyloid. The ROS detection using nanosensors in single cells has gained increasing attention. Traditional fluorescent dyes have a number of disadvantages. Here, we have developed an electrochemical method for ROS detection in single living neurons treated by different beta-amyloids (beta-amyloid$_{42,}$ isomerized Asp$_7$ beta-amyloid and phosphorylated Ser$_8$ beta-amyloid) We have previously reported the fabrication of disk-shaped carbon nanoelectrodes based on a quartz nanopipette that were functionalized by platinum for improvement of their electroactive properties. We incubated neuronal cells with different beta-amyloids for 4 hours and measured the content of ROS inside the single cells using a platinum nanoelectrode. For the first time, it was shown that after exposure to various amyloids, the ROS level within cells is different. Isomerized Asp7 beta-amyloid turned out to be the most toxic beta-amyloid.

This work was supported by the Russian Science Foundation № 19-74-10059.

IMMEDIATE POSTTRAUMATIC SEIZURES PREDICT EARLY BUT NOT LATE MORTALITY IN RATS

Chizhova O.A.[1], Komoltsev I.G.[1,2], Frankevich S.O.[1,2], Volkova A.A.[1], Shirobokova N.I.[1], Salyp O.Yu.[1], Bashkatova D.A.[1], Shalneva D.V.[1], Kostyunina O.V.[1], Kostryukov P.A.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Department of Functional Biochemistry of Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

olesya_chj@mail.ru

Introduction. Traumatic brain injury (TBI) is a leading cause of death, chronic headache, symptomatic epilepsy, and early dementia worldwide. It can be hypothesized that immediate posttraumatic seizures may be associated with secondary brain damage and increase mortality risks after TBI. In this study we aimed to reveal components of immediate seizures potentially predicting acute or late mortality in rats.

Methods. We used a lateral fluid percussion brain injury model (LFP) in male Sprague-Dawley rats (n=26). Immediate posttraumatic seizures were analyzed. We measured total duration of seizure as well as duration of running, clonic and tonic movements. The duration of apnea, dyspnea and hypopnea were recorded and signs of cyanosis in extremities, nose bleedings were noticed. Righting reflex, ability to maintain position and sensitivity to pain were registered. Mortality was assessed during 3 months after LFP.

Results. Total duration of convulsion ($p=0.002$), duration of secondary convulsion ($p=0.007$), and restoration of left righting reflex ($p=0.019$) predicted acute mortality. Presence of motor symptoms ($p=0.013$), laying moves ($p=0.047$), hypertonus of paws ($p=0.029$), and secondary convulsions ($p=0.02$) was also associated with acute mortality. Nose bleeding was one of the accurate symptoms associated with acute mortality ($p<0.001$). Reanimation ($p=0.047$) and cyanosis ($p=0.002$) also predicted acute mortality. There was no difference between seizures in rats died during late posttraumatic period and survivor rats.

Conclusion. We found that immediate posttraumatic seizures predict acute mortality but none of them predicts late mortality. Muscle contractions may indicate prolonged excitation in the brain, leading to excitotoxicity and secondary brain damage. Nose bleeding may be a sign of neurogenic pulmonary edema. Brain edema, activation of the sympatho-adrenal system and brainstem herniation may be involved pathogenesis of acute death.

Supported by RSF, grant № 22-25-00713

IMPROVING EFFECT OF INTRANASAL INSULIN, C-PEPTIDE AND THEIR COMBINATION ON HORMONAL STATUS AND HYPOTHALAMIC SIGNALING IN RATS WITH TYPE 1 DIABETES MELLITUS

Zorina I.I., Derkach K.V., Bakhtyukov A.A., Basova N.E., Shpakov A.O.

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Sciences, St. Petersburg, Russia

alex_shpakov@list.ru

Acute type 1 diabetes mellitus (DM1) with severe insulin deficiency, acute hyperglycemia and hyperphagia leads to impaired incretin and adipokine status, endocrine dysfunctions and altered hypothalamic regulation. This is largely due to brain insulin deficiency, and the use of intranasally administered insulin (I-I), which compensates for this deficiency, can prevent a number of negative DM1 consequences. Since in DM1 there is also a deficiency of proinsulin C-peptide, which can enhance insulin effects, it seems promising to use I-I with intranasally administered C-peptide (I-CP). The aim was to study the effect of 7-day treatment of rats with short-term DM1 induced by streptozotocin (65 mg/kg) with I-I (20 μg/rat/day), including in combination with I-CP (36 μg/rat/day), on the blood levels of hormones and incretins and the gene expression of food intake factors, receptors and the regulators of mitochondrial dynamics and apoptosis in the hypothalamus. I-I and its combination with I-CP to varying degrees restored the levels of leptin, glucagon-like peptide-1, thyroid-stimulating and luteinizing hormones and thyroid hormones, all reduced in DM1, and also normalized the level of ghrelin, which was elevated in DM1, with little effect on testosterone levels. One of the mechanisms of this is the I-I-induced increase in the gene expression of hypothalamic M4-melanocortin receptor and pro-opiomelanocortin, decrease in the expression of the orexigenic neuropeptide Y gene, and normalization of gene expression of proteins responsible for mitochondrial dynamics (*Mfn-1*, *Mfn-2*, *Drp-1*), apoptosis (*Bcl-2*) and autophagy (*Beclin-1*). Monotherapy with I-CP was ineffective and had little effect on the assessed parameters. Thus, I-I and its combination with I-CP restore the hormonal and incretin status, functioning of the endocrine system and hypothalamic signaling in male rats with acute DM1.

The work was supported by the state assignment AAAA-A18-118012290427-7.

INHIBITION OF GLUCOCEREBROSIDASE ACTIVITY LEADS TO INCREASED RELEASE OF EXTRACELLULAR VESICLES

Kulabukhova D.G.[1,2], Nikolaev M.A.[1,2], Garaeva L.A.[1], Baydakova G.V.[3], Zakharova E.Y.[3], Emelyanov A.K.[1,2], Pchelina S.N.[1,2]

1 - NRC «Kurchatov Institute» - PNPI, St. Petersburg, Russia
2 - Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russia
3 - Research Center for Medical Genetics

kulabuhova_dg@pnpi.nrcki.ru

Lysosome functionality is critical for the regulation of extracellular vesicles (EVs) secretion and content. An inhibition of lysosomal function with different alkaline agents increase EVs secretion. Mutations in the glucocerebrosidase gene (*GBA*) are considered as a high-risk factor for Parkinson's disease (PD). Molecular mechanism of PD pathogenesis remains unknown but the EVs role in transmission of alpha-synuclein pathogenic species is widely discussed. The aim of our work is to assess GBA dysfunction on EVs secretion.

Methods. An inhibition of GBA activity was achieved via SH-SY5Y cells incubation with 100 μM of selective inhibitor of glucocerebrosidase, conduritol-β-epoxide (CBE), for 3 days. Lysosomal disfunction was generated via cells incubation with 50 nM bafilomycin A1. The levels of enzymatic activity of galactosylceramidase (GALC), alpha glucosidase (GAA), alpha galactosidase (GLA), glucocerebrosidase (GBA), acid sphingomyelinase (ASM) and alpha-l-iduronidase (IDUA) were measured by liquid chromatography-tandem mass spectrometry in dried cells spots. EVs were isolated from 50 ml the supernatant of SH-SY5Y cells by sequential centrifugation and evaluated by nanoparticle tracking analysis (NTA).

Result. In SH-SY5Y cells treated with bafilomycin A1 the enzyme activity of all enzymes (GALC, GAA, GLA, ASM, IDUA and GBA) was decreased on 86,02±13,08%, as in case of CBE only GBA-activity was decreased (20,2 vs 0,01 M/l/hour ($p = 0,0001$)). The incubation of cells with bafilomycin A1 increases the number of EVs in ten times ((34,1±1,2)E10vs(3,98±0,4)E10 particles/ml. Decrease in GBA activity also leads to moderate increase in the number of EVs (5,49±0,1)E12vs(8,18±0,5)E12 particles/ml, $p=0,002$ or $p=0,001$ respectively).

Conclusion. Our data provide for the first time an evidence that GBA dysfunction lead to an increase of EVs secretion, that could influence on pathogenesis of PD, associated with mutations on *GBA* gene.

The reported study was funded by RFBR, project number 19-315-90059.

INVESTIGATION OF THE THERAPEUTIC POTENTIAL OF THE CHAPERONE INDUCER U133 IN A MODEL OF THE PRECLINICAL STAGE OF PARKINSON'S DISEASE IN AGED RATS

Pazi M.B.[1], Belan D.V.[1], Polonik S.G.[2], Ekimova I.V.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia
2 - Elyakov Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia

pazimariia@gmail.com

Introduction. Parkinson's disease (PD) is a chronic neurodegenerative disorder of mostly elderly patients. PD remains incurable due to late diagnosis and symptomatic therapy since our understanding of the etiology and pathophysiology of PD is limited. The disease is characterized by cardinal motor symptoms and a range of non-motor symptoms. One of the most frequent and clinically significant neuropsychiatric disorders of PD is depression, which may precede motor disturbances. To date, there are no effective neuroprotectors and antidepressants suitable for PD treatment. In the present study, we evaluated the antidepressant and neuroprotective effects of preventive therapy by chaperone inducer U-133 in a model of the preclinical stage of PD in aged rats.

Methods. The model of PD was created by intranasally administered proteasome inhibitor lactacystin (LC) to Wistar rats (20 months). The echinochrome derivative U-133 was administered intraperitoneally thrice: 4 hours after each LC injection and 7 days after the last LC injection. Sucrose preference test, methods of immunohistochemistry and biochemistry were used in the research.

Results and discussion. It was shown that therapy with U-133 prevents the manifestation of signs of anhedonia in the model of the preclinical stage of PD, indicating an antidepressant-like effect of U-133. The effect was associated with an increase in the content of Hsp70 (*HSPA1*) protein and weakening of the pathological hallmarks of PD (neurodegeneration, α-synuclein pathology and neuroinflammation) in the mesolimbic dopaminergic reward system and noradrenergic locus coeruleus system of model rats. We believe that the antidepressant-like effect of U-133 is based on the ability of Hsp70 to trigger a multifactorial mechanism of powerful neuroprotection, which promotes restoration of the function of ascending emotiogenic monoaminergic pathways.

Conclusion. Results indicate the therapeutic significance of Hsp70 induction for the prevention of emotional disorders and PD-like neurodegeneration.

The work was carried out within the state assignment (AAAA18-118012290427-7).

IONOTROPIC GLUTAMATE RECEPTORS AND CALPAINS IN F^- TOXICITY IN RAT HIPPOCAMPUS

Nadei O.V., Agalakova N.I.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St.Petersburg, Russia

olganadej@gmail.com

Although fluorine was recognized as one of ten chemicals of major public health concern (WHO), molecular mechanisms of F^- neurotoxicity are studied poorly. Since F^- is known to increase intracellular Ca^{2+} content, its action on the CNS cells can be linked with excessive Ca^{2+} influx through AMPA and NMDA receptors channels which induces excitotoxic responses partly mediated by activation of Ca^{2+}-dependent proteases calpains. Likewise, calpains are known to exert multiple effects on glutamatergic synapses. Present study was designed to evaluate the effects of excessive F^- consumption on expression of AMPA and NMDA receptors and calpains in hippocampal cells of Wistar rats.

The animals consumed water with 0.4 (control), 5, 20 and 50 ppm F^- (NaF) for 12 months. The efficiency of learning and memory formation was evaluated by novel object recognition and Morris water maze tests. Protein and mRNA content of AMPARs, NMDARs and calpains in hippocampus were detected by immunoblotting and RT-PCR, respectively.

In hippocampal cells F^- intake increased Gria1gene and GluA1 protein expression, resulting in enhanced ratio between Ca^{2+}-permeable and -impermeable AMPARs in membranes. The number of GluN1 and GluN2B subunits of NMDARs was unaltered, whereas Grin2a mRNA and GluN2A protein content increased. F^- consumption led to stimulation of calpain-1 and its effectors - RhoA GTPase, PHLPP1 phosphatase and ERK1/2 kinase. In contrast, the expression of calpain-2 and its substrates (phosphatase PTEN and kinase mTOR) was stable.

These results confirm a causative role of F^- in adverse cognitive capacities of rats associated with elevated GluA1/GluA2 and GluN2A/GluN2B ratios leading to excessive Ca^{2+} influx and disturbances in synaptic processes. Besides, overstimulation of calpain-1 with unaltered activity of calpain-2 can indicate the disruption between early and late LTP phases.

The study was performed within the state assignment of Federal Agency of Scientific Organizations of Russia (theme No AAAA-A18-118012290371-3).

LPS-INDUCED ACCUMULATION OF LIPID DROPLETS IN PC12 CELLS: THE ROLE OF DOWNREGULATION OF CPT-1 EXPRESSION AND FATTY ACIDS OXIDATION

Parnova R.G., Nikolaeva S.D., Fock E.M.

Sechenov Institute of Evolutionary Physiology & Biochemistry of the Russian Academy of Sciences St. Petersburg, Russia

rimma_parnova@mail.ru

It is well-known that inflammation and oxidative stress in different kind of cells trigger the formation of cytosolic lipid droplets in which fatty acids (FA) are sequestered in the form of triacylglycerides (TAG) and cholesterol esters. This metabolic shift is critical in protection of cells from lipotoxicity or lipid peroxidation. In nervous system, different types of glial cells could form lipid droplets whereas little is known whether neurons alter their neutral lipid metabolism by such a way in response to pathological stimuli. Using PC12 cells, a rat neuronal pheochromocytoma cell line widely explored in neuroinflammation-related studies, we examined the effect of LPS on lipid droplets formation focusing on the underlying mechanisms. Our previous work has shown that PC12 cells express TLR4 and respond to LPS by ROS production associated with the decrease of cell viability. Incubation of PC12 cells with LPS for 24 h led to significant accumulation of cytosolic lipid droplets and increase of TAG content. To understand the metabolic origin of TAG accumulation, we pre-incubated PC12 cells with [^{3}H]-oleic acid before challenge with LPS, and then examined the incorporation of the radioactive label into the main lipid classes. LPS caused an increase in radioactivity in TAG and free oleic acid accompanied by significant decrease in [^{3}H]-oleic acid oxidation. The observed effects of LPS were mimicked by etomoxir, inhibitor of carnitine palmitoyltransferase-1 (CPT-1), the rate-limiting enzyme for the β-oxidation of FA. Treatment of PC12 cells for 24 h with LPS reduced the expression of CPT-1 protein. The data obtained evidence that under inflammatory stimulus PC12 cells are able to stimulate formation of lipid droplets which is probably triggered by ROS production and mitochondrial disfunction. CPT-1-mediated decrease of FA oxidation and sequestration of excess free FA into TAG appears to be an important mechanism of LPS-induced lipid droplets accumulation in PC12 cells.

LABEL-FREE 3D MAPPING OF OXYGEN AND ROS OF SINGLE LIVING NEURON CELLS

Erofeev A.S.[1], Gorelkin P.V.[1], Vaneev A.N.[1], Korchev Y.E.[1,2]

1 - National University of Science and Technology "MISiS", Moscow, Russia
2 - Imperial College London, London, UK

erofeev.as@misis.ru

Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells.

Additionally, nanopipette probes still hold great promises as intracellular biosensors.

Here we describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5-200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of neuron cell. These novel platinum nanoelectrodes are useful for understanding cell oxygen metabolism and can be employed to study the redox biochemistry and biology of cells, tissues and organisms.

Pt nanoelectrodes used for intracellular ROS measurements of control cells and cells with Aβ42 aggregates. We noticed significant increase in ROS related on the ability of Aβ42 to disrupt the mitochondrial level and induce oxidative stress. Higher cytotoxicity of amyloid aggregates than free oligomers can be assumed which was questionable in various studies.

The reported study was funded by Russian Science Foundation RSF grant 19-79-30062

LACTOBACILLUS METABOLIC PRODUCTS STIMULATE THE STORE-DEPENDENT CALCIUM ENTRY IN PC-12 WITH SWEDISH DOUBLE MUTATION

Sobol C.V.

Institute of Evolutionary Physiology and Biochemistry, St.Petersburg, Russia

peep9@yandex.ru

It is known that store-dependent calcium entry significantly suppressed in Alzheimer's disease (Putney, 2003). The attenuation of the store-dependent calcium entry in Alzheimer's disease leads to an increase in the Aβ42 synthesis (Putney, 2000). An increase Ca2+ entry under the add-back conditions significantly reduces Aβ (Dreses-Werringloer et al., 2008).

It was shown that Lactobacillus metabolic products (PP) significantly stimulates the store-

dependent entry of Ca2+ in PC-12, neurons and in PC-12 with Swedish double mutation (K670M/N671L). It is not ruled out that stimulation of capacitative Ca2+ entry with PP can lead to decrease Aβ generation.

On the other hand, alterations in hippocampal neurogenesis can be considered as an integral part of Alzheimer's disease (Maruszak et al., 2014; Oh et al., 2015). Earlier, we showed that PP promoted neuritogenesis and neuronal differentiation in pheochromocytoma (PC12) cells (Sobol et al., 2005; Sobol and Belostotskaya, 2005).

Therefore, PP can be considered as natural product for possible prevention of neurodegeneration.

This work was supported by a state contract with the Federal Agency for Scientific Organizations of Russia (project no. AAA-A18-118012290371-3)

LITHIUM INTERFERES TRICYCLIC ANTIDEPRESSANTS ACTION ON NMDA RECEPTORS

Sibarov D.A., Boikov S.I., Stepanenko Y.D., Antonov S.M.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St.Petersburg, Russia

dsibarov@gmail.com

N-methyl-D-aspartate receptors (NMDARs) are an essential target for the analgetic action of tricyclic antidepressants (TCAs) against neuropathic pain. Using the patch-clamp method for recording whole-cell transmembrane currents in cortical neurons of primary cultures, we observed two components of TCA action on NMDARs. These components could be characterized as the open-channel block and the calcium-dependent inhibition. The "pure" channel block of NMDARs by TCAs is calcium-independent and occurs at concentrations vastly exceeding the therapeutic concentrations of 0.5-1.5 μM measured in the human plasma. At therapeutic concentrations, however, amitriptyline and desipramine produce calcium-dependent inhibition of NMDAR currents. Removing extracellular calcium or binding of intracellular calcium with BAPTA abolishes inhibition of NMDARs by TCAs within this particular range of concentrations. This type of inhibition resembles an enhancement of NMDAR calcium-dependent desensitization (CDD) caused by KB-R7943 - an inhibitor of the sodium-calcium exchanger (NCX). NCX, as known, extrudes the calcium entering via activated NMDARs and counteracts NMDARs CDD. In agreement the substitution of lithium (NCX substrate inhibitor) for sodium in the external solution enhances CDD of NMDARs. Therefore lithium suppresses calcium-dependent effects of amitriptyline and

desipramine on NMDARs and the IC50 of this lithium effect is about ~3 mM. Thus NCX inhibitors probably weaken NMDARs mediated analgetic effects of amitriptyline and desipramine. It is likely that NCX and the CDD of NMDARs represent promising targets to treat neuropathic pain pharmacologically.

The study was supported by RSF grant 21-15-00403.

LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN BRAIN PHOSPHOLIPIDS: WHAT FUNDAMENTALLY NEW WE LEARN BY STUDYING INSECTS?

Parnova R.G.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

rimma_parnova@mail.ru

In most vertebrates the composition of C20-22 omega-3 PUFAs in brain PLs is similar to the high prevalence of C22:6ω3. In mammals, during prenatal development, it accumulates in the brain and activates neurogenesis, neurite outgrowth and formation of synaptic contacts enhancing neuronal and synaptic plasticity. A dietary deficiency of omega-3 PUFAs impairs brain and vision development. Due to these facts, there is a strong idea that for the normal functioning of the brain neuronal membranes must contain C22:6ω3. Also, it is claimed that C22:6ω3 is an obligatory component of all known photoreceptor systems - from algae to humans. However, literature and our data indicate that the highly developed brain of insects does not contain C22:6ω3 and has a low content of PUFAs longer than C18. Even in the photoreceptors, only C18:2 and C18:3 have been detected among PUFAs. Exceptions are aquatic species, which accumulate a significant amount of C20:5ω3 produced by microalgae.

According to our data, in cockroaches, the main PUFA in brain PLs is C20:4ω 6 with traces of omega-3 PUFAs, in locusts - C18:3ω3 with no PUFAs longer than C18, in amphibiotic dragonflies - C20:5ω3. These data indicate that the fundamental mechanisms of the CNS functioning, common to all animals, can be provided by a different set of PUFAs.

Ontogenesis of dragonflies is accompanied by a significant complication of their behavior and sensory information processing. Adult dragonflies were the first invertebrates to demonstrate the brain's ability to create internal models providing unique hunting strategies. Our data indicate that, compared with the aquatic larvae, not only the amount of PLs is increased in the brain of adults, but also the content of C20:5ω3 is almost 4 times higher. These data suggest that the "cognitive role" of omega-3 PUFAs were used in evolution long before the appearance of mammals.

MATERNAL HYPERHOMOCYSTEINEMIA DISTURBES LONG-TERM POTENTIATION AND HIPPOCAMPAL-RELATED COGNITIVE FUNCTIONS IN YOUNG RATS

Vasilev D.S., Postnikova T.Yu., Tumanova N.L., Dubrovskaya N.M., Kalinina D.S., Shcherbitskaia A.D., Zaitsev A.V.

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, RAS, St. Petersburg, Russia

dvasilyev@bk.ru

Maternal hyperhomocysteinemia (HHC) is one of the common complications of pregnancy that causes offspring cognitive deficits during postnatal development. The effects of high homocysteine levels were extensively studied during postnatal life, however, little is still known about mechanisms of its deleterious action in prenatal period. Thus, we investigated the effects of maternal HHC on synaptic properties in the hippocampus and hippocampal-related cognitive functions in young rats. HHC was induced in pregnant rats by administration of methionine (0.6mg/kg) in drinking water. In the entorhinal cortex and hippocampus of HHC rat pups during the first month after birth we observed a reduction in the number of pyramidal neurons and increased number of glial cells compared to controls. At the ultrastructural level there were an increased number of undeveloped growth cones during first two weeks, and decreased number of developed synapses, especially axo-spinal ones during first month after birth. We found that 20-days-old HHC rats had a decrease in hippocampal long-term synaptic potentiation. These alterations were accompanied by a significant decline in the level of synaptopodin protein as well as the number of synaptopodin-positive dendritic spines in the CA1 area of the hippocampus was reduced in the HHC rats. These changes resulted in significant learning and memory deficits: HHC pups demonstrated worst performance in a Morris water maze and novel object recognition test. Supported by RFBR-20-015-00388 and Russian state budget assignment 075-00408-21-00.

MECHANISMS OF AMPA RECEPTORS INHIBITION BY PHENYTOIN

Dron M.Yu., Zhigulin A.S., Tikhonov D.B., Barygin O.I.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St Petersburg, Russia

Neuro.mike@yahoo.com

Phenytoin is a long-standing, anti-seizure drug widely used in clinical practice. The main target for phenytoin in the brain, which is believed to underlie its anticonvulsant properties, are voltage-gated sodium channels. We performed a screening for activity against native calcium-permeable AMPA receptors (CP-AMPARs) and calcium-impermeable AMPA receptors (CI-

AMPARs) among zonisamide, lamotrigine, levetiracetam, tiagabin, topiramate, carbamazepine, felbamate, vigabatrin, gabapentin and phenytoin at 100 μM concentrations using the whole-cell patch-clamp method on isolated Wistar rat brain neurons. Phenytoin, unlike to all other substances, inhibited both major AMPA receptor subtypes, being much more active against CI-AMPARs (IC50=30±4 μM) than against CP-AMPARs (250±60 μM), so we have shown for the first time that phenytoin is more active against CI-AMPARs. Among known AMPAR antagonists, similar preference for CI-AMPARs demonstrated pentobarbital. Phenytoin of 100 μM was drastically more active in the absence than in the presence of cyclothiazide (74 ± 4% vs. 21 ± 7% inhibition, respectively; $p < 0.001$). The inhibitory effect of 50 μM pentobarbital was also significantly attenuated in the presence of cyclothiazide. Also it was shown that phenytoin trapping is dependent on kainite concentration: 300 μM demonstrated pronounced trapping at 100 μM kainite, but trapping was questionable at 500 μM kainite. Phenytoin has a hydantoinic ring, which potentially could underlie in blocking of calcium-impermeable AMPARs. Surprisingly, 5-benzylhydantoin, hydantoin, primidon and ethosuximide demonstrated only weak inhibition at 300 μM concentration. So, close 3D similarity between phenytoin and pentobarbital suggests a common binding site in the pore and mechanism of inhibition. Taking all mentioned data into account, phenytoin inhibitory activity on AMPA receptors may contribute to its antiepileptic properties as well as its side effects.

The reported study was funded by RFBR, project numbers 16-04-01080, 20-34-90039.

MECHANISMS OF NMDA RECEPTOR INHIBITION BY AMIDINE AND GUANIDINE COMPOUNDS

Zhigulin A.S., Barygin O.I.

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

arseniy.zhigulin@yandex.ru

Many pharmaceuticals inhibit N-methyl-D-aspartate (NMDA) receptors as side targets. In the present work we studied the action of the serine protease inhibitors nafamostat, gabexate and camostat, and an antiprotozoal compound, furamidine, on native NMDA receptors in rat hippocampal pyramidal neurons. These compounds contain one or two amidine or guanidine groups in their structure. Nafamostat, furamidine and gabexate inhibited NMDA receptors at −80 mV holding voltage with IC_{50} of 0.20±0.04, 0.64±0.13 and 16±3 μM, respectively, whereas camostat was ineffective. The action of nafamostat and furamidine was voltage-dependent, while gabexate demonstrated practically voltage-independent inhibition. Nafamostat and furamidine demonstrated tail currents, suggesting a ‘foot-in-the-door’ mechanism of action; gabexate did not show any signs

of ‘foot-in-the-door’ or trapping channel block. Gabexate action was also non-competitive, suggesting allosteric inhibition of NMDA receptors. The structures of nafamostat and furamidine are rather rigid and elongated and their molecules cannot fold into more compact forms. The molecule of previously studied diarylamidine compound diminazene has similar properties, and it also demonstrated a ‘foot-in-the-door’ mechanism. By contrast, the gabexate molecule can fold, but its folded structure differs drastically from that of typical NMDA receptor blockers, in agreement with its voltage-independent inhibition. These findings provide a better understanding of the structural determinants of NMDA receptor antagonism. These data may help to support the repurposing of nafamostat and related compounds for the treatment of neurodegenerative diseases, including glaucoma. However, additional studies on the effects of these compounds on synaptic transmission in brain slices and on neuroprotective activities in animal disease models are needed to establish possible clinical applications.

This work was supported by the Ministry of Education and Science of Russia (NCMU, agreement No. 075-1502020-916).

MECHANISMS OF HIPPOCAMPAL VULNERABILITY TO STRESS UNDERLIE COMORBIDITY BETWEEN NEUROLOGICAL AND PSYCHIATRIC DISORDERS

Gulyaeva N.V.[1,2]

1 - Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 - Research and Clinical Center for Neuropsychiatry of Moscow Healthcare Department, Moscow, Russia

nata_gul@ihna.ru

The data reported during last three decades suggest that dysfunction of the hypothalamic-pituitary-adrenocortical (HPA) axis and excessive cortisol secretion are common features of many brain diseases, including focal brain injuries (stroke and traumatic brain injury), cognitive disorders/dementias, epilepsy, and depressive disorders. Clinical findings demonstrating augmented release of glucocorticoids are supported by the results in experimental models. Glucocorticoid signals are mediated through their receptors which are widely presented in the brain, being most abundant in the limbic systems, in particular in the hippocampus, a brain region key to learning, memory, and emotions. Excessive glucocorticoid signaling disrupts the function and impairs the structure of the hippocampus as well as hippocampus-associated neural networks. Selective vulnerability of the hippocampus to various stressful factors is mediated by the reception of glucocorticoid hormones secreted during stress and represents a price of high functional plasticity

and pleiotropy of this limbic structure. HPA axis dysfunction shared by common neurological and mental diseases becomes the basis for their comorbidities, the hippocampus being a nodal point. Shared molecular and cellular mechanisms stimulated by glucocorticoids include the dysfunction of glucocorticoid receptors, neurotransmitter systems, and neurotrophic factors, development of neuroinflammation, leading to neurodegeneration and loss of hippocampal neurons, as well as disturbances in neurogenesis in the subgranular neurogenic niche and formation of aberrant neural networks. These glucocorticoid-dependent processes are associated with altered adaptive plasticity and the development of chronic stress-related comorbid pathologies, in particular, stroke/depression and temporal lobe epilepsy/depression.

Supported by Russian Science Foundation [project # 20-65-47029].

MECHANISMS OF NEUROTOXICITY OF PRENATAL HYPERHOMOCYSTEINEMIA

Arutjunyan A., Milyutina Yu., Shcherbitskaia A.

Ott Institute of Obstetrics, Gynecology, and Reproductive medicine, St. Petersburg, Russia

alexarutjunian@gmail.com

Based on the experimental studies, it was hypothesized that in addition to a direct neurotoxic action on the fetal brain, prenatal hyperhomocysteinemia (PHH) has a negative effect on the formation of the fetal and newborn nervous systems by changing the functional state of the placenta. Under the influence of PHH (model of methionine loading of pregnant rats), an increase in the level of neurotrophins (BDNF and NGF) precursors in the fetal brain and placenta was shown. An increase in the content of BDNF and NGF precursors is associated with a violation of their processing to mature forms, in contrast to which proBDNF and proNGF have a depressing effect on brain maturation in early ontogenesis. It is assumed that changes in neurotrophins processing, along with oxidative stress (OS) and the inflammatory process initiated by it, as well as apoptosis, play an important role in disorders of brain development in offspring after PHH.

The data showing the increase in the content of the proinflammatory cytokine IL-1β in the cortex of the offspring, who underwent PHH, together with histological studies, suggest that neuroinsflammatory processes play an important role in pathogenesis of PHH. Disorders of brain development in early ontogenesis associated with the development of neuroinflammation may be long-term. The results of morphological studies of the hippocampus and neocortex of rats that have undergone PHH indicate a violation of neuronal cell migration, a lag in the development of nervous tissue, an increase in the number of degenerating neurons and the number of glial cells on the 5th and 20th days of postnatal development. It is established that some changes in OS indicators that

were manifested after PHH persist until puberty. Along with this PHH induces the disturbances of cognitive functions in sexually mature offspring.

Supported by RFBR 18-015-00099 and Russian state budget assignment 1021062812133-0-3.2.2.

MECHANISMS OF SOUND-INDUCED OPENING OF THE BLOOD-BRAIN BARRIER

Blokhina I.A.[1], Karandin G.A.[1], Semyachkina-Glushkovskaya O.V.[1], Kurths J.G.[1,2,3]

1 - Saratov State University, Saratov, Russia
2 - Humboldt University, Physics Department, Berlin, Germany
3 - Potsdam Institute for Climate Impact Research, Potsdam, Germany

inna-474@yandex.ru

The blood-brain barrier (BBB) is a highly selective barrier, which controls the penetration of blood-borne agents into the brain or the release of metabolites and ions from the brain tissue to blood. Therefore, the BBB plays a vital role in central nervous system (CNS) health protecting the brain against pathogens and toxins. The limitations of our knowledge about the nature of BBB explain the slow progress in the therapy of brain diseases and absence of methods for drug delivery to the brain in clinical practice. In our study on rats, we demonstrate that a factor such as loud sound (100 dB 370 Hz), which we can meet in daily life when listening to MP3/MP4 players or at a rock concerts, reversibly opens the BBB to low- and high-molecular-weight molecules. We also discuss mechanisms underlying the sound related opening of BBB - loud sound reversibly opens the BBB via stress-mediated TJ machinery disorganization. Our data are consistent with the hypothesis suggesting an important role of stress in the BBB opening via mechanisms underlying epinephrine-induced enhancement of the BBB permeability. This opens an informative platform for novel fundamental knowledge about the nature of BBB and for the development of a noninvasive brain drug delivery technology.

This work was supported by grant from the RF Governmental grant 075-15-2019-1885.

MECHANISMS OF STRUCTURAL REORGANIZATION OF THE HIPPOCAMPUS AND INFERIOR COLLICULI DURING EPILEPTOGENESIS IN KRUSHINSKY-MOLODKINA RATS

Kulikov A.A., Glazova M.V., Aleksandrova E.P., Chernigovskaya E.V.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

alexey.kulikov@iephb.ru

Krushinsky-Molodkina (KM) rats are a genetic model of audiogenic epilepsy. The triggers of the acute audiogenic seizure (AGS) are the auditory brainstem structures (inferior colliculi), while repetitive AGSs (audiogenic kindling (AK)) spread epileptiform discharges through limbic structures (hippocampus) that can be considered as the model of epileptogenesis. KM rats start to demonstrate a stable AGS only after the age of 3 months. We investigated 2 different models of epileptogenesis. Firstly, we studied a structural reorganization of hippocampus and inferior colliculi during development of audiogenic epilepsy from P15 to P120. Secondly, we studied a structural reorganization of hippocampus at different stages of AK.

We showed that at early stages of postnatal development of KM rats hippocampus and inferior colliculi were characterized by significantly increased activity of ERK1/2 kinase that was accompanied with increased proliferation and apoptosis in comparison with control Wistar rats. Also these structures had a smaller cell population. We showed an aberrant migration of newborn cells to the hilus. These 2 processes were also typical for adult KM rats.

In other experiments we exposed KM rats to AK of different durations (4, 14, and 21 AGS). AK of different durations led to stimulation of proliferation, abnormal migration, and glutamatergic differentiation of new neurons both in the DG granular layers and hilus. AK also stimulated sprouting of mossy fibers and enhanced expression of synaptopodin in the hippocampus indicating generation of new synaptic contacts between granular cells, mossy cells, and CA3 pyramidal neurons. Data revealed 2 waves of cell death in the hippocampus at initial and at late stages of AK, autophagy activated only in initial stages of AK.

Thus, our data suggest that aberrant neurogenesis, and accelerated glutamatergic differentiation of new born neurons, mossy fiber sprouting in KM rats contribute to reorganization of hippocampal network during different stages of epileptogenesis.

MEDIUM-CHAIN TRIGLYCERIDE-INDUCED KETOSIS AFFECTS HOUSEKEEPING AND GLUTAMATE TRANSPORT GENE EXPRESSION IN THE RAT BRAIN IN REGION-SPECIFIC MANNER

Nikitina V.A.[1], Schwarz A.P.[2,1], Krytskaya D.U.[1], Shcherbakova K.P.[1], Trofimov A.N.[1]

1 - Laboratory of Neurobiology of the Brain Integrative Functions, Institute of Experimental Medicine, St. Petersburg, Russia
2 - Laboratory of Molecular Mechanisms of Neuronal Interactions, Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St.Petersburg, Russia

v.a.n1kitina@yandex.ru

Mild intermittent ketosis established by ingestion of medium-chain-triglycerides (MCT) is known to elicit many neuroprotective and cognition-enhancing effects, although the exact mechanisms in the brain are still largely unknown. In this study we established mild intermittent ketosis in adult Wistar rats by adding MCT oil (2 ml/kg, i.g., during 6 h of fasting) to standard chow for 1 month (water was used as control). We then measured the mRNA expression of 9 housekeeping genes (*Actb*, *B2m*, *Gapdh*, *Hprt1*, *Pgk1*, *Ppia*, *Rpl13a*, *Sdha*, *Ywhaz*), often used as reference, in the medial prefrontal cortex (mPFC), dorsal (DH) and ventral hippocampus (VH), by RT-qPCR and used the RefFinder® online tool to analyze the reference gene stability of each gene in each region. Using the three most stable genes as reference, we assessed the mRNA expression of less stable housekeeping genes and *EAAT2* (glutamate transporter 1) in each region. The examined reference gene panel showed the lowest stability in DH, compared to VH and mPFC. As reference genes, *Ppia*, *Actb*, and *Rpl13a* were the most stable in mPFC; *Rpl13a*, *Ywhaz*, and *Pgk1* were the most stable in DH; *Ywhaz*, *Sdha*, and *Ppia* were the most stable in VH. Using the three most stably expressed reference genes in each region, we found that the *Gapdh* mRNA was upregulated, while the *Sdha* mRNA was downregulated by the MCT treatment in mPFC, but not in VH or DH. *EAAT2* was upregulated in DH and downregulated in VH of the MCT-fed animals. These results demonstrate that 1 month of supplementation with MCT affects mRNA expression of genes involved in energy metabolism and glutamate transport in the rat brain in region-specific manner, and also once again highlights the necessity to select the most adequate reference genes for RT-qPCR analysis.

Supported by Russian Science Foundation (RSF, project no. 19-75-10076).

MEMBRANE CATALYSIS IN INTERACTION OF THREE-FINGER TOXINS WITH NICOTINIC ACETYLCHOLINE RECEPTORS

Shenkarev Z.O.[1,2], Chesnokov Y.[3], Zaigraev M.M.[1,2], Chugunov A.O.[1], Kulbatskii D.S.[1], Kocharovskaya M.V.[1,2], Paramonov A.S.[1], Bychkov M.L.[1], Shulepko M.A.[1], Kamyshinsky R.[3], Yablokov E.O.[4], Kirpichnikov M.P.[1,5], Lyukmanova E.N.[1,2,5]

1 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia
2 - Phystech School of Biological and Medical Physics, Moscow Institute of Physics and Technology (National Research University), Dolgoprudny, Moscow Region, Russia
3 - National Research Center "Kurchatov Institute", Moscow, Russia
4 - Institute of Biomedical Chemistry, Moscow, Russia
5 - Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology», Faculty of Biology, Lomonosov Moscow State University, Moscow, Russia

zakhar-shenkarev@yandex.ru

Nicotinic acetylcholine receptor of α7 type (α7-nAChR) presented in the nervous and immune systems and epithelium is a promising therapeutic target for cognitive disfunctions and cancer treatment. WTX is a non-conventional three-finger neurotoxin from Naja kaouthia venom, targeting α7-nAChR with weak affinity. There is no data on interaction mode of non-conventional neurotoxins with α7-nAChR. Using α-bungarotoxin (classical three-finger neurotoxin with high affinity to α7-nAChR), we showed applicability of cryo-EM to study interactions of α7-nAChR extracellular ligand-binding domain (α7-ECD) with its ligands. Cryo-EM structure of the α7-ECD/WTX complex together with NMR data on membrane active site in the WTX molecule and mutagenesis data allowed to reconstruct the α7-nAChR/WTX structure in the membrane environment. WTX interacts at the entrance to the orthosteric site located at the receptor intersubunit interface and simultaneously forms the contacts with the membrane surface. WTX interaction mode with α7-nAChR significantly differs from α-bungarotoxin's one, which do not contact the membrane. Our study provides evidence of the 'membrane catalysis' mechanism for non-conventional neurotoxins.

This study made under financial support of Russian Science Foundation (project #19-74-20163).

The work was supported by the Russian Science Foundation (Project 19-74-20163).

METABOLIC MECHANISMS OF NEUROINFLAMMATION AS A TARGET FOR THE PREVENTION AND POTENTIAL CORRECTION OF CENTRAL INSULIN RESISTANCE

Komleva Y.K.[1,2], Khilazheva E.D.[1,2], Potapenko I.V.[2], Panina Y.A.[1,2], Chernykh A.I.[2], Salmina A.B.[2,3], Lopatina O.L.[1,4], Gorina Y.V.[1,2], Shuvaev A.N.[2]

1 - Department of Biochemistry, Medical, Pharmaceutical & Toxicological Chemistry, Krasnoyarsk State Medical University named after Prof. V.F. Voino-Yasenetsky, Krasnoyarsk, Russia
2 - Research Institute of Molecular Medicine and Pathobiochemistry, Krasnoyarsk, Russia
3 - Laboratory of Experimental Brain Cytology, Division of Brain Sciences, Research Center of Neurology, Moscow, Russia
4 - Shared Research Center for Molecular and Cellular Technologies, Krasnoyarsk, Russia

yuliakomleva@mail.ru

Alzheimer's disease (AD) is a most common neurodegenerative disorder that leads to dementia. Recently, much attention has been paid to the study of the NLRP3 multiprotein complex as a possible target molecule in the treatment of many conditions. This study was devoted to the understanding of the potential therapeutic roles of the NLRP3 inflammasome in neurodegeneration occurring concomitant with brain insulin resistance. Methods: We studied the role of NLRP3 inflammasomes in health and neurodegeneration in maintaining brain insulin signaling using behavioral, electrophysiological approaches, immunohistochemistry, ELISA and real-time PCR. Results: NLRP3-dependent mechanisms have been demonstrated in the amygdala in normal conditions and in neurodegeneration. We have confirmed, that the basal level of NLRP3 expression is necessary for neurogenesis processes, regulating mainly the early stages: cell proliferation and differentiation. Our data suggest that expression of NLRP3 inflammasomes in neural stem cells and neuroblasts may contribute to stimulation of adult neurogenesis in physiological conditions. It has been shown that NLRP3 inflammasomes are required for insulin-dependent glucose transport in the brain and memory consolidation. However, *Nlrp3* knockout protects mice from the pathological effects of beta-amyloid oligomers and protects against the development of insulin resistance, which is manifested by the unchanged level of expression of IRS1-Ser compared to the control. It has been experimentally proven that preventing the development of local insulin resistance by blocking NLRP3 inflammasomes and mediated neuroinflammation should be considered a pathogenetically grounded approach to correcting cognitive disorders in Alzheimer's disease. Conclusion: Taken together, our data revealed the protective role of *Nlrp3* deletion in the regulation of fear memory and the development of Aβ-induced neurodegeneration, providing a novel target for the clinical treatment of this disorder.

This work was supported by a grant from the President of the Russian Federation for State support of young Russian scientists - Doctor of Science MD-2368.2022.3.

MODULATION OF INHIBITORY PROCESSES IN THE BRAIN BY SYNTHETIC PHOTOSWITCHABLE COMPOUNDS

Petukhova E.O.[1], Ponomareva D.N.[1,2,3], Bregestovski P.D.[2,3]

1 - Institute of Neurosciences, Kazan State Medical University, Kazan, Russia
2 - Department of Normal Physiology, Kazan State Medical University, Kazan, Russia
3 - Institut de Neurosciences des Systèmes, Aix-Marseille University, INSERM, INS, Marseille, France

petukhovaeo@mail.ru

Photopharmacology aims to develop compounds whose biological activity can be switched by light. Herein, we investigated two compounds, Glyght and Azo-NZ1, designed to modulate activity of GABA and/or glycine receptors. Both carry an azobenzene unit, which allows the light-induced *cis/trans* isomerization of the compounds.

The compounds were tested on glycinergic and GABAergic evoked inhibitory postsynaptic currents (eIPSCs), recorded from mouse hypoglossal motor neurons and dentate gyrus granule cells in the «whole cell» configuration using patch-clamp technique. Diode emitting UV (365 nm) was used to induce *trans*-to-*cis* photoisomerization, blue (455 nm) and/or visible light were applied for reverse isomerization.

Azo-NZ1 significantly suppressed the GABAergic and the glycinergic eIPSCs in a photoswitchable manner. The *trans* isomer of Azo-NZ1 (*trans*-Azo-NZ1, 100 μM) reduced the GABAergic eIPSCs amplitude to 57.9±3.4% (P21-28, $p<0.05$, n=6), while the *cis*-Azo-NZ1 caused restoration to 73.9±6.4% ($p<0.05$). The amplitude of glycinergic eIPSCs reduced to 61.5±4.7% (P4-P8, $p<0.05$, n=8) in presence of *trans*-Azo-NZ1 (15 μM), and increased to 80.5±4.2% ($p<0.05$) after switching it to the *cis*-configuration. Previous study on the heterologously expressed glycine receptors (GlyRs) demonstrated that the *trans*-Azo-NZ1 effectively blocks "foetal" GlyRs formed by alpha2 subunits, but not "adult" GlyRs formed by alpha1 subunits. The complete blockage of the glycinergic eIPSCs could not be achieved on brain slices even at high concentrations of Azo-NZ1 (up to 300 μM), suggesting the presence of currents mediated by alpha1 GlyRs.

Analysis of Glyght (100μM) showed that in the *cis*-configuration it causes a decrease of the amplitude of glycinergic eIPSCs to 60.9±3.3% (P2-P6, $P<0.05$, n=7); in the *trans*-configuration the compound was ineffective. Glyght didn't affect GABAergic eIPSCs (n=8).

Thus, (i) Azo-NZ1 is an effective tool for photoswitchable modulation of eIPSCs mediated by $GABA_A$ and alpha2 GlyRs; (ii) Glyght is the specific light-controlled modulator of glycinergic eIPSCs.

Supported by the RSF grant: 18-15-00313.

MONITORING AND TARGETING OF THE ASTROGLIAL ACTIVITY IN THE BRAIN

Salmina A.B.[1,2], Khilazheva E.D.[2], Morgun A.V.[2], Teplyashina E.A.[2], Boytsova E.B.[2], Gorina Ya.V.[2], Komleva Yu.A.[2], Mosyagina A.I.[2], Malinovskaya N.A.[2], Kharitonova E.V.[2], Shuvaev A.N.[2]

1 - Research Center of Neurology, Moscow, Russia
2 - Prof. V.F. Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia

allasalmina@mail.ru

It is generally accepted that astrocytes coordinate neuronal excitability, buffer extracellular ions, affect mitochondrial neuronal activity, control development and pruning of synapses, and support adult neurogenesis. Particularly, astrocytes are required for adequate energy production in neuronal cells, adjustment of local blood flow to neuronal needs in active brain regions, and regulation of blood-brain barrier (BBB) integrity. Contribution of astrocytes to the regulation of neuroplasticity or BBB permeability is provided by coordinated release of gliotransmitters, cytokines, and metabolites, thereby suggesting novel opportunities for pharmacological and non-pharmacological modulation. Recently, glia becomes to be a promising target for manipulating the brain plasticity in (patho)physiological conditions. Deciphering the key cellular, molecular and biochemical mechanisms underlying complex neuron-glia or glia-endothelial interactions, and accurate monitoring of astroglial activity coupled to brain functions are necessary for the development of high-efficacy methods of diagnostics and therapy. Achievements in optogenetics and neurophotonics are new challenges in studying and manipulating with astroglial activity.

We applied optogenetics-based protocols for monitoring and targeting the astroglial activity within the neurovascular unit and BBB in physiological and pathological conditions (experimental Alzheimer's disease). In the original in vitro neurogenic niche/BBB models, we found that target activation of ChR2-expressing astrocytes affects mitochondrial dynamics and mitochondrial activity in astroglial cells and brain microvessel endothelial cells, stimulates neurogenesis and controls developmental fate of neural stem cells and neuronal progenitor cells, modulates proliferative capacity of brain microvessel endothelial cells. These effects are partially mediated by the release of lactate from activated astrocytes, its intercellular transport via monocarboxylate transporters and action at GPR81 lactate receptors.

The study was supported by the State Assignment for the Research (Ministry of Public Health, 2018-2020) and the Grant of the President of the Russian Federation for the support of the Leading Scientific Teams (НШ 2547.2020.7, 2020-2021).

MORPHOLOGICAL AND FUNCTIONAL CHANGES IN THE HIPPOCAMPUS OF YOUNG RATS DURING THE LATENT PHASE OF THE LITHIUM-PILOCARPINE MODEL OF TEMPORAL LOBE EPILEPSY

Postnikova T.Yu., Diespirov G.P., Amakhin D.V., Soboleva E.B., Zaitsev A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St.Petersburg, Russia

tapost2@mail.ru

Status epilepticus (SE) causes persistent abnormalities in the functioning of neuronal networks, often resulting in worsening epileptic seizures. Many details of cellular and molecular mechanisms of seizure-induced changes are still unknown. In this work, using the lithium–pilocarpine model in three-week-old rats, we examined the morphological and electrophysiological changes in the hippocampus within a week following pilocarpine-induced seizures. We found that almost a third of the neurons in the hippocampus and dentate gyrus died on the first day, but this was not accompanied by impaired synaptic plasticity at that time. A diminished long-term potentiation (LTP) was observed following three days, and the negative effect of SE on plasticity increased one week later, being accompanied by astrogliosis. The attenuation of LTP was caused by the weakening of N-methyl-D-aspartate receptor (NMDAR)-dependent signaling. NMDAR-current was more than two-fold weaker during high-frequency stimulation in the post-SE rats than in the control group. Application of glial transmitter D-serine, a coagonist of NMDARs, allows the enhancement of the NMDAR-dependent current and LTP restoration. These results suggest that the disorder of neuron–astrocyte interactions plays a critical role in the impairment of synaptic plasticity.

This study was supported by the Russian Science Foundation, grant № 21-15-00430.

NCGC607 CHAPERONE BINDS TO THE MUTANT GLUCOCEREBROSIDASE IN ALLOSTERIC SITES AND RESTORES ITS ACTIVITY IN PRIMARY MACROPHAGES AND IPSC-DERIVED DOPAMINERGIC NEURONS FROM PATIENTS WITH PARKINSON'S DISEASE

Kopytova A.[1], Rychkov G.[1,2], Grigoreva E.[3], Cheblokov A.[1], Pavlova S.[3], Sorogina D.[3], Drozdova E.[3], Nikolaev M.[1,4], Izymchenko A.[1], Bogdanova D.[1], Ibatullin F.[1], Baydakova G.[5], Miliukhina I.[4,6], Medvedev S.[3], Zakian S.[3], Zakharova E.[5], Pchelina S.[1,4], Emelyanov A.[1,4]

1 - NRC «Kurchatov Institute» - PNPI, Gatchina, Russia
2 - Peter the Great St.-Petersburg Polytechnic University, St.Petersburg, Russia
3 - Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia
4 - First Pavlov State Medical University of St. Petersburg, St.Petersburg, Russia
5 - Research Center for Medical Genetics, Moscow, Russia
6 - N.P. Bechtereva Institute of the Human Brain, RAS, St.Petersburg, Russia

kopytovaalena@mail.ru

Background: Mutations in the *GBA1* gene, encoding the lysosomal enzyme glucocerebrosidase(GCase), cause Gaucher disease(GD) and are the most common genetic risk factor of Parkinson's disease(PD). Pharmacological chaperones(PCs) can bind and stabilize mutant enzymes, improve lysosomal trafficking and activity. NCGC607 is one of the most promising PC, however further studies are needed to identify the involved binding sites(BSs) on the GCase surface and verify its potency to restore GCase activity.

Objective: To identify and characterize BSs on the GCase surface suitable for NCGC607 and to test the potency of NCGC607 to restore GCase activity in primary macrophages and dopaminergic neurons(DA) derived from induced pluripotent stem cells(iPSC) from GBA1-PD patients.

Methods: Potential BSs were identified using Molsoft ICM software. Binding modes of NCGC607 in identified sites were assessed by molecular docking and molecular dynamics simulations techniques. Mononuclear fraction was isolated from whole blood of four GBA1-PD patients with «mild» mutation(N370S), two GBA1-PD patients with «severe»(L444P) and three healthy controls. Primary macrophages and iPSC-derived DA neurons were cultured as described earlier (KopytovaA et al.,ParkinsonismRelatDisord.2021.10;84:112-121; DrozdovaE et al. I International Forum of Genomic and Biomedical Technologies.2021.SURGUT) with 4μM of NCGC607. GCase enzymatic activity was measured by LC-MS/MS.

Results: On the GCase surface we have identified and characterized using molecular modeling methods six potential allosteric BSs matching NCGC607. Only BS1, BS2 and BS3 can be considered as potential targets for NCGC607. We showed that NCGC607 treatment significantly increased GCase activity both in primary macrophages and in iPSC-derived DA neurons from GBA1-PD patients with «mild» *GBA1* mutations by 1,4- and 1,5-fold (p=0.028 and p=0.046,

correspondingly).

Conclusions: Our study demonstrated potential allosteric BSs matching NCGC607 and confirmed its ability to increases GCase activity in primary macrophages and iPSC-derived DA neurons from GBA1-PD patients with «mild», but not «severe» mutations.

The study was funded by RSF №22-25-00721

NEUROCHEMISTRY OF CREATIVITY – WHAT DO WE KNOW?

Nagornova Zh.V., Beljakov G.V., Galkin V.A., Vasenkina V.A., Grokhotova A.V., Shemyakina N.V.

Sechenov Institute of evolutionary physiology and biochemistry, Russian academy of sciences, St. Petersburg, Russia

nagornova_zh@mail.ru

The impact of different neurotransmitter systems on human creativity might be considered either by assessing the polymorphism of transmitter regulation genes in persons with high and low creative abilities, or by assessing the impact of drugs and stimulants. There were attempts to find a relationship between dopamine and creativity. Patients with Prakinson's disease treated with dopaminergic drugs demonstrated enhanced verbal and visual creativity (Faust-Socher et al., 2014) and experienced bursts of creativity (Walker et al., 2006, Canesi et al., 2012) as compared to neurologically healthy controls. However it seems that relationships between dopamine and creativity differs from person to person (Käckenmester et al., 2019), phenomenon of creativity bursts in patients that receive dopaminergic replacement therapy is questionable (Salvi et al., 2021), in some studies were obtained no influence of dopamine agonists on cognitive flexibility (Smyth et al., 2007) as element of creativity. Tyrosine is known to be a precursor of catecholamines, but in a double-blind, placebo-controlled, randomized cross-over study, tyrosine intake to improve divergent thinking was not well established (Colzato et al., 2014). There is no clear answer of extra dopamine and its precursors intake influence on creativity and there should be further studies with reproducible methodological approaches. Some data describe noradrenergic influence on creativity. Cognitive flexibility test results during stress were improved with propranolol (beta-adrenergic antagonist) administration (Alexander et al., 2007). Propranolol improves, compared to placebo, the ability to solve cognitive anagram tests (Zamzow et al. ,2016, Campbell et al.,2007). In autism spectrum disorder studies it was found that propranolol administration benefited cognitive flexibility to access semantic and associative networks (Beversdorf et al., 2011) in autists. Thus, there are various data of psychoactive substances modulating noradrenergic and dopaminergic systems influence on different aspects of creativity and it is the theme for the further clarification.

The work is supported by the IEPhB Research Program 075-0152-22-00

NEUROTROPIC AND CRYOPROTECTIVE EFFECTS OF L-CARNOSINE

Mokrushin A.A.

Pavlov Institute Physiology, St. Petersburg, Russia

mok@inbox.ru

Dipeptide carnosine (β-alanyl-L-histidine) was found in significant quantities in the mammalian brain, especially in the olfactory structures. Because it can penetrate the blood-brain barrier, easily and has not side effects, the therapeutic potentials of carnosine to some brain disorders have attracted great attention. The objectives of the study were the elucidation neurotropic, neuroprotective and neuroprotective effects of *L*-carnosine on the activity of the glutamatergic (α-amino-3-hydroxy-5-methylisoxalol-4-propionic acid (AMPA) и N-methyl-D-aspartate (NMDA) receptors and $GABA_B$-ergic mechanisms in olfactory cortex slices.

The presynaptic and postsynaptic components of extracellular potentiatials: the compound action potential of the lateral olfactory tract, AMPA and NMDA EPSP, and inhibitory postsynaptic potential (IPSP) were investigated in olfactory cortex slices during application of *L*-cfrnosine in different concentrations. Nootropic effects of *L*-carnosine have been studied in models of non-associative learning – a long-term posttetanic potentiation/depression (LTP/LTD). Neuroprotective effects of *L*-carnosine have been explored during cryopropreservation before and after the deep freezing (-10^{o}C) and subsequent rewarming of brain slices.

L-carnosine in the range of concentrations of 5-20 mM did not induce changes in the activities of AMPA and NMDA-dependent mechanisms, but increased the activity of $GABA_B$-ergic mechanisms. In the model of non-associative learning *L*-carnosine promoted the development of LTP, but inhibited the development of LTD. Pretreatment of slices using L-carnosine (20 mkM) before freezing prevented excessive swelling of slices after subsequent rewarming and contributed to preservation of the activity of the AMPA and NMDA mechanisms. Results of the study argue for a significant protective potential of endogenous dipeptide *L*-carnosine.

NEW PROSPECTS FOR GLIOMA THERAPY: LYMPHATIC DELIVERY OF LIPOSOMES TO BRAIN TISSUE

Khorovodov A.[1], Semyachkina-Glushkovskaya O.V.[1,2], Fedosov I.V.[1], Shirokov A.A.[1,3], Vodovozova E.L.[4], Alekseeva A.[4], Blokhina I.A.[1], Terskov A.V.[1], Evsyukova A.S.[1], Karandin G.A.[1], Elovenko D.D.[1], Adushkina V.V.[1], Dubrovsky A.I.[1], Ageev V.[1], Telnova V.V.[1], Tsven A.[1], Sokolovski S.G.[5], Rafailov E.U.[5], Kurths J.[1,6]

1 - Saratov State University, Saratov, Russia
2 - Humboldt University, Berkin, Germany
3 - Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Science,Moscow, Russia
4 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, UK
5 - Optoelectronics and Biomedical Photonics Group, AIPT, Aston University, Birmingham
6 - Potsdam Institute for Climate Impact Research, Potsdam, Germany

khorovodov2012@yandex.ru

The blood-brain barrier (BBB) not only plays an important role in the protective function of the central nervous system (CNS), but is also the main obstacle to the effective delivery of drugs to the brain in various CNS pathologies, including oncology. The development of safe methods for an effective delivery of medications and nanocarriers to the brain can be a revolutionary step in the overcoming this limitation. Here, we studied the lymphatic delivery of GM1 liposomes to the brain and fluorescent glioma in rats, by passing the BBB. Our results clearly demonstrate that the deep cervical lymph nodes are an anatomical platform for a unique connection between the brain and the peripheral lymphatic network. Indeed, the introduction of liposomes and test indicators into deep cervical lymph nodes was accompanied by their delivery to the meninges, brain parenchyma, and glioma.

The research was supported by the RF Government grant 20-15-00090.

NITROGEN OXIDE AND HYDROGEN SULFIDE IN BRAIN OF RATS WITH PRENATAL HYPERHOMOCYSTEINEMIA

Yakovleva O.V., Belova Yu.I.

Kazan Federal University, Kazan, Russia

a-olay@yandex.ru

Hyperhomocysteinemia (Hcy) is a systemic metabolic disease caused by abnormalities in homocysteine metabolism. It is known that in adult rats with prenatal Hcy, a decrease in H2S synthesis is observed. There is also evidence in the literature that homocysteine inhibits the synthesis of NO. The aim of our work was to correlate the content of nitric oxide and hydrogen

sulfide in the brain of rats with prenatal Hcy in different age periods.

The animals were divided into two equal groups: 1) control, rats from females kept under standard animal conditions (n=25); 2) experimental, rats from females 2 weeks before and during pregnancy on a methionine diet (n=25). Using the spectrophotometric method, the content of NO metabolites and H2S ions in the brain of rats was analyzed.

In the brain of control rats during the first month of life, we observed the minimum concentration of NO metabolites (12.9±2.6 μM, n=10). Then there was an increase in the concentration of NO metabolites to 22.9±2.5 μM by the 3rd month of life (n=10, $p<0.05$). In animals of the experimental group, the content of NO metabolites in the brain was 22.6±2.5 μM, which is significantly higher than the control values (n=15, $p<0.05$). At the age of 90 days, the concentration of NO metabolites increased to 36.8±3.4 μM, which was significantly higher than the control values (n=10, $p<0.05$). Analysis of the concentration of HS- ions revealed a decrease in the content of HS- ions in the brain tissues of the experimental group (7.9±0.8 μM vs. 11.5±0.6 μM, n=10, $p<0.05$) as in animals of the first month of life, and the third month (8.0±1.3 μM versus 12.0±1.1 μM, n=15, $p<0.05$). Thus, the increase in oxidative stress at prenatal Hcy may be one of the reasons for the increase in the synthesis of NO metabolites and the decrease in free HS- ions in the brain of rats.

The work was carried out within the framework of the Russian Science Foundation grant no. 20-15-00100.

NMDA RECEPTOR INHIBITION BY ANTIHISTAMINE COMPOUNDS

Dron M.Yu., Barygin O.I.

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St Petersburg, Russia

Oleg_Barygin@mail.ru

Antihistamine compounds are a class of drugs used to treat symptoms of allergies. Besides histamine receptors, some of them can affect other targets in the organism, including NMDA type ionotropic glutamate receptors. Sedative properties of several first generation antihistamine compounds might be partially explained by NMDA receptor inhibition. Therefore, we decided to perform electrophysiological screening for activity against NMDA receptors among several antihistamine compounds: diphenhydramine, antazoline, tripellenamine (first generation), cetirizine, loratadine, desloratadine, ketotifen (second and third generation). Experiments were performed on isolated rat hippocampal CA1 pyramidal neurons using whole cell patch clamp technique. Except for cetirizine and ketotifen, other compounds demonstrated significant activity against NMDA

receptors; The IC50 values at -80 mV holding voltage in the absence of magnesium ions were 1.8 ± 0.4 μM for antazoline, 13 ± 3 μM for desloratadine, 19 ± 4 μM for loratadine, 42 ± 8 μM for diphenhydramine, and 65 ± 17 μM for tripellenamine. The data for antazoline and diphenhydramine are in line with previous results, while inhibitory activity of loratadine and desloratadine was demonstrated by us for the first time. NMDA receptor inhibition by antazoline and diphenhydramine was voltage-dependent. In addition, they could be trapped in the closed NMDA receptor channels, evidencing channel block as the main molecular mechanism of inhibition. In contrast, loratadine and desloratadine did not demonstrate characteristical signs of channel block, and further studies are needed to determine their main molecular mechanisms of NMDA receptor inhibition.

The reported study was funded by RSF grant 17-74-10117 and by RFBR grant 20-34-90039.

NA,K-ATPASE α1-SUBUNIT IS A BETA-AMYLOID RECEPTOR

Petrushanko I.[1], Barykin E.[1], Tverskoi A.[1], Petrovskaya A.[1], Strelkova M.[1], Leonova O.[1], Anashkina A.[1], Tolstova A.[1], Adzhubei A.A[1], Bogdanova A.[2], Makarov A.[1], Mitkevich V.[1]

1 - Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia
2 - Institute of Veterinary Physiology, Vetsuisse Faculty and the Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland

mitkevich@gmail.com

Earlier we showed that beta-amyloid 1-42 ($A\beta_{42}$) interacts with Na,K-ATPase, inhibits its hydrolytic and transport activity and triggers activation of Src kinase in micromolar doses. This study proves the hypothesis that Na,K-ATPase is a receptor for $A\beta_{42}$ in nanomolar concentrations, as it is for the cardiotonic steroids (CTS). The colocalization of $A\beta_{42}$ with Na,K-ATPase, and between the enzyme and Src kinase in SH-SY-5Y neuroblastoma cells has been proved using immunocytochemistry and proximity ligation assay. Treatment of cells with 100 nM $A\beta_{42}$ causes release of Src kinase from the cytosolic domain of the Na,K-ATPase α1-subunit, but does not alter its transport activity. Activation of Src kinase in response to the $A\beta_{42}$ interaction with Na,K-ATPase has been observed in neuroblastoma cells and in Na,K-ATPase:Src kinase protein mixtures exposed to $A\beta_{42}$. Inhibitor of the Src kinase interaction with the Na,K-ATPase, NaKtide, blocked the activation of Src kinase by $A\beta_{42}$. Activation of Src kinase by sub-inhibitory concentrations of $A\beta_{42}$ induced Ca^{2+}-independent free radical production and shift in thiol redox state in neuroblastoma cells. The stimulatory effect of $A\beta_{42}$ on Src kinase was lost under hypoxic conditions. The obtained data indicate that, at nanomolar doses, $A\beta_{42}$ uses Na,K-ATPase as a receptor, triggering Src kinase-dependent signaling in the brain cells similar to that CTS induce in peripheral tissues. It could be a

part of adaptive, protective and physiologically relevant signaling mechanisms in the brain.

Acknowledgments: the research was supported by the RSF Grant 19-74-30007

NEUROINFOVIEWER: A SOFTWARE PACKAGE FOR ANALYSIS OF MINISCOPE DATA

Erofeev A.I.[1], Barinov D.S.[2], Gerasimov E.I.[1], Pchitskaya E.I.[1], Bolsunovskaja M.V.[2], Vlasova O.L.[1], Bezprozvanny I.B.[1,3]

1 - Laboratory of Molecular Neurodegeneration, Institute of Biomedical Systems and Biotechnology, Graduate School of Biomedical Systems and Technologies, St. Petersburg State Polytechnical University, St. Petersburg, Russia
2 - Laboratory of Industrial Data Streaming Systems, National Technology Initiative Center for Advanced Manufacturing Technologies, St. Petersburg State Polytechnical University, St.Petersburg, Russia
3 - Department of Physiology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, USA

alexandr.erofeew@gmail.com

A miniscope is a miniature fluorescence microscope developed by Mark Schnitzer's laboratory. It is used for fluorescence imaging of neural activity in free-moving animals, and is actively used in neuroscience research. The average weight of the standard miniscope is about 3 g. A flexible coaxial cable is used for power supply, control, and video transfer to a computer. The data obtained as a result of a miniscope recording experiment comprise a video file that contains a time-lapse recording of changes in fluorescence of a reporter, usually a calcium (Ca^{2+})-sensitive indicator, such as genetically encoded calcium indicator (GCaMP). These changes in fluorescent signal reflect changes in neuronal activity in the field of view, and various methods are used to process miniscope data and to extract information about neuronal activity. The methods for the initial processing of miniscope data include semi-manual region of interest (ROI) analysis, principal component analysis (PCA) and independent component analysis (ICA) analysis, and automated software packages, such as constrained nonnegative matrix factorization (CNMF), constrained nonnegative matrix factorization for microendoscopic data (CNMF-E), and 1-photon-based calcium imaging signal extraction pipeline (MIN1PIPE). Initial processing of miniscope data is followed by a high-level analysis that aims to identify neuronal networks, describe brain activity changes during behavioral tasks, and detect changes in brain activity caused by neurodegeneration, epilepsy, stroke, and other pathological conditions. However, few tools have been developed for high-level analysis of miniscope data. Calculations used to quantify correlation matrices of neuronal activity based on miniscope data have been described but the tools used for calculating correlation matrices in these studies are not publicly available. For this reason, we present the NeuroInfoViewer (NIV) analysis

tool, which is useful for high-level analysis of miniscope data.

The miniscope data processing consists of the following sequence of steps:

1. Conversion of avi raw miniscope imaging data files to tiff format.
2. Initial processing of generated tiff files by CNMF-E, MIN1PIPE, or similar software.
3. Export of information about time-lapse activity of individual neurons, as a set of csv (comma-separated values) files.
4. Import of resulting csv files to NIV, to calculate and visualize pairwise correlations between neuronal activity traces.
5. Export of correlation matrices from NIV in csv format, for further analysis.

The first step in data analysis is to export neuronal activity traces in csv format. The script code for export depends on the initial miniscope data processing algorithm. As an example, we developed a MATLAB language script for exporting neuronal activity traces from CNMF-E. This script can be downloaded from https://github.com/nxt007/NIV/tree/main/scripts. Examples of csv files generated using this script can be found in the https://github.com/nxt007/NIV/tree/main/test_data/CNMF-E_demo folder. The metadata file that is generated as a result of executing this script has a filename that ends with meta.csv (which we write as *_meta.csv). The file contains the following components: (1) data format, (2) paths to files containing information about calcium traces and locations of neurons, (3) information about video frame rate, (4) the original frame rate (in fps) of the video sequence.

The main data file *_*meta.csv* is imported into NIV by using the *Import csv* command. From imported neuronal activity traces, the NIV application is able to calculate and visualize pairwise correlations between neuronal activity traces and generate correlation matrices. NIV consists of two matlab files: *NIV.mlapp* (MATLAB main application source file) and *useviewneuron2.m* (single neuron information display function). All data processing is handled by the *DataUpdate* function. After loading the data, neuronal activity information is displayed. Depending on the selected algorithm, one of three neuron interactivity coefficient (NIC) functions is executed. Additional NIC algorithms can easily be integrated if necessary. The first implemented algorithm, *NonZeroSpikeFramesIntersec*, produces a visual representation of correlations in neuronal activity. This algorithm uses preprocessed neuronal activity (spike) data. For the pair comprising the *i*-th and *j*-th neurons, neuron interactivity coefficient is defined as the ratio of the total of the *i*-th neuron activity frames to the intersection of the simultaneous *i*-th and *j*-th neurons' activity frames. The second implemented algorithm, *ThresWarmCool*, is more complex. This algorithm uses a raw calcium trace as input and produce states trace with two possible values: active and inactive. The parameters of the algorithm, which should be adjusted for each experiment, include the following parameters: *Threshold*: the triggering threshold value; *Warm* (warm-up period): the time (number of

frames) during which the spike value for a frame must exceed the threshold to switch to the *Active* state; *Cool* (cool-down period): the time (number of frames) during which the spike value for a frame must be below the threshold to switch to the *Inactive* state. The third implemented algorithm, *SignalCrossCorrMax*, uses the MatLab signal cross-correlation function.

When the pairwise correlations between the neurons calculated, it is possible to export the following files from NIV: three images—neuron heatmap (Heatmap), neuronal activity correlations pairs, and neuronal activity correlations heatmap —in *.fig* and *.png* format, and the pairwise correlation matrix in *csv* format. Examples of export files generated using NIV can be found at https://github.com/nxt007/NIV/tree/main/test_output.

A software package, NIV, which calculates and visualizes correlations between neuronal activity traces recorded in miniscope imaging experiments, was developed. The current version of NIV (0.8.2) allows the user to import data from a single imaging experiment, perform correlation analysis, and generate results in either a graphical form or as a csv matrix file. The NIV code was deposited in the public domain to facilitate its use by the neuroscience community. In subsequent versions of NIV, we are planning to add an option to compare neural activity from several experiments, using the CellReg algorithm.

The research was funded by the Ministry of Science and Higher Education of the Russian Federation as part of the World-class Research Center program: Advanced Digital Technologies (contract No. 075-15-2020-934 to IB).

NEUROCHEMICAL AND BEHAVIORAL ASPECTS OF CHEMOGENETIC MODULATION OF NEURONAL ACTIVITY IN THE BRAIN REGIONS

Dygalo N.N.[1,2], Drozd U.S.[1,2], Bulygina V.V.[1], Kalinina T.S.[1,2], Sukhareva E.V.[1,2], Komysheva N.P.[1], Shishkina G.T.[1], Lanshakov D.A.[1,2]

1 - Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
2 - Novosibirsk State University, Novosibirsk, Russia

dygalo@bionet.nsc.ru

Effects of glutamatergic neuron activity in the prefrontal cortex, dorsal hippocampus and amygdala on the expression of the neuronal activation (c-Fos) and neuroplasticity (BDNF) markers and acute stress-induced behaviors were studied using chemogenetic approach. For this, AAVs encoding activating - CaMKIIa-hM3D(Gq)-mCherry, inhibiting - CaMKIIa-hM4D(Gi)-mCherry DREADDs or control - CaMKIIa-Egfp vectors were injected bilaterally into the brain structures of adult male rats. One month later and 30 minutes after stimulation of DREADDs with clozapine-N-

oxide, anxiety and depression-like behaviors of the rats were assessed in the light/dark box (LDB) or tail suspension (TST) tests. Brain tissue samples for real-time RT-PCR and immunohistochemistry were collected an hour after TST. mCherry and Egfp fluorescence and mRNAs levels for DREADDs confirmed expression of vectors in the targeted regions. Cortical activation and inhibition of hippocampus attenuated anxiety in the LDB. Expression of Gq in the hippocampus and cortex reduced immobility in TST. Hippocampal Gi, on the contrary, increased immobility, and cortical Gq overcame this effect. Gi in amygdala stimulated anxiety while Gq increased active behavior in TST. Hippocampal Gq reduced c-fos mRNA level in this structure. Cortical Gq induced a decrease, whereas Gi an increase of c-fos mRNA in the amygdala. Hippocampal Gi decreased cortical bdnf mRNA. The decreases in the levels of c-fos mRNA in the hippocampus and bdnf mRNA in the cortex by hippocampal Gi were leveled both by cortical Gi or Gq. The bdnf mRNA in the amygdala increased with a decrease in the hippocampal neurons activity and cortical Gi enhanced this effect. Amygdala Gq upregulated bdnf mRNA in the cortex, and both c-fos and bdnf mRNAs in the midbrain. Thus, chemogenetic disruption of activities and interregional connectivity of the interacting brain structures is capable of altering gene expression and behavioral manifestations of the psycho-emotional state.

Supported by RFBR 20-015-00129, RSF 19-15-00093

NEUROGENESIS AFTER SINGLE PTZ-INDUCED SEIZURE IN MICE IS NOT ACCOMPANIED BY NEUROINFLAMMATION IN THE HIPPOCAMPUS

Aniol V.A., Ivanova-Dyatlova A.Yu., Gulyaeva N.V.

Institute of higher nervous activity and neurophysiology RAS, Moscow, Russia

aniviktor@yandex.ru

Generalized seizures are usually followed by increased neurogenesis in the dentate gyrus (DG). New neurons, generated after such seizures often demonstrate atypical features , e.g. abnormal axon branching (mossy fibers sprouting), altered polarity of new neurons (hilar apical dendrites), and migration of new neurons into the hilus. However, these changes usually develop in parallel with neuronal cell loss and neuroinflammation in the hippocampus, which are typical for pilocarpine and kainate models. On the contrary, seizures induced by a single pentylenetetrazole (PTZ) injection are not accompanied by cell loss, therefore we used this model to verify if single seizure itself without cell loss can provoke changes to adult neurogenesis in the DG.

We injected cell proliferation marker 5-bromo-2'-deoxyuridinre (BrdU) in CD-1 mice at different times points after the PTZ injection (70 mg/kg). The most evident increase (1.5-fold) in

proliferation of cells in the DG was registered on the 3rd day after the seizure. Also, two weeks after the seizure respective 1.5-fold increase in the number of doublecortine-positive (neuronal differentiation marker) cells was found.This increase was determined by involvement of new resting stem-like cells since no effect of seizure was observed if BrdU was injected before the seizure. Most cells became neurons 3 months later; however, no difference could be found at this time point between the groups, indicating that all excessive neurons generated after the seizures eventually die.

In contrast to severe models like pilocarpine- or kainate-induced seizures, we found no significant increase in inflammatory cytokines level in the hippocampus. Moreover, the level of some cytokines (e.g. TNF-alpha) demonstrated the transient decrease soon after the seizures. Also, neither microglial nor astrocytic activation could be seen in the hippocampus at any time point after the seizure.

NEURONS AS PRODUCERS OF THE PERINEURONAL NETWORK KEY COMPONENTS IN SPINAL CORD INJURY

Sabirov D.[1], Kabdesh I.[1], Davletshin E.[1], Mukhamedshina Y.[1,2]

1 - Kazan (Volga Region) Federal University, Kazan, Russia
2 - Kazan State Medical University, Kazan, Russia

davraniwe@gmail.com

Chondroitin sulfate proteoglycans (CSPGs) are a major group of anionic glycoproteins that form a barrier to axonal growth and neuronal plasticity after spinal cord injury (SCI). One of the major group of CSPGs that inhibit axon elongation is the hyaluronan-binding CSPG family of lecticans, including aggrecan (ACAN), neurocan (NCAN), brevican (BCAN) and NG2. This study aimed to analyze the expression of organizing and stabilizing extracellular matrix molecules at different distances from the SCI epicenter and posttraumatic periods. We have shown differences in the relative expression of mRNA of genes encoding the synthesis of lecticans: BCAN, NCAN, versican (VCAN) and NG2 proteoglycan in both acute (7 dpi) and chronic (30 dpi) periods of SCI at different distances from the epicenter of damage. Using immunohistochemical method we registered shifts in ACAN expression in the population of plate IX neurons expressing calcium-binding protein parvalbumin (PARV+ neurons) and in the population of neurons simultaneously expressing NeuN and choline acetyltransferase (ChAT) in SCI. Against the background of no shift in the number of preserved PARV+ neurons in the anterior columns near the damage area (4 mm), the number of PARV+/ACAN+ neurons increased at 7 and 30 days, which is also observed at 7 and

11 mm. At the same time, ACAN expression in PARV+/ACAN+ neurons at all distances from the epicenter remains elevated for at least 30 days. The results of immunohistochemical study confirm the possibility of ACAN expression previously shown not only in astrocytes, which are the main producer of lecticans in norm and pathology, but also in neurons with high spike activity. These data allow us to consider neurons as producers of key components of the perineuronal network in neuroregeneration.

This study was supported by a grant from the Russian Science Foundation (Grant Number 21-75-00016; YM).

NEUROPROTECTION OF CAMP-PATHWAY ACTIVATION INFLUENCES Ca2+ RESPONSES AND MULTI-KINASE SIGNALING CAUSED BY HOMOCYSTEINE NEUROTOXICITY

Ivanova M.A., Abushik P.A., Sibarov D.A., Antonov S.M.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

etrnlrddl@gmail.com

Activation of cyclic adenosine monophosphate (cAMP) synthesis is known to lead triggering of neuroprotective signal cascades. The goal of our investigation was to evaluate the influence of forskolin, an activator of adenylate cyclase, and its induced cAMP production on the neurotoxic action of L-homocysteine (HCY) in cortical rat neurons in primary cultures. Neurotoxic effects of HCY (100 μM), agonist-evoked intracellular calcium (Ca2+) responses and changes in mitochondrial membrane potential were studied using fluorescence detection methods. The analysis of neuronal viability showed that long-term (24 h) action of HCY caused neuronal apoptosis and necrosis that was followed by a decrease of quantity of live cells. Activation of adenylate cyclase by forskolin (1 μM presence in the external solution) prevented apoptosis and necrosis of neurons during long-term treatment with HCY exhibiting neuroprotective properties. As for the cytotoxic action, in experiments on neurons loaded with Fluo-3, HCY caused an increase of the intracellular Ca2+ concentration. A short-time treatment with forskolin significantly decreased Ca2+ influx into neurons during HCY action. Additional analysis of the developmental dynamics of mitochondrial dysfunction on HCY exposure showed that forskolin can prevent the decrease in mitochondrial membrane potential (ϕmit, Rhodamine123 approach), which is usually observed in excitotoxic stress. Our experiments with a protein kinase A (PKA) inhibitor, the protein kinase C (PKC) inhibitor chelerythrine, and the calmodulin-dependent kinase II (CaMKII) inhibitor KN93 demonstrated that the neuroprotective effect of forskolin on long-term exposure to HCY involves

PKA and CaMKII. We also studied whether the expression of proteins involved in apoptotic pathways and anti-apoptotic proteins was affected by forskolin during 4 h neurotoxic action of HCY. Proteins such as caspase-3, p53, and BAX, which contribute to the caspase-dependent apoptotic pathway, were decreased during action of forskolin. The expression of apoptosis-inducing factor (AIF) was also evaluated together with Bcl-2, which antagonizes AIF release from mitochondria. In conclusion, the results demonstrate an important role of adenylate cyclase and cAMP in the regulation of the Ca2+ accumulation in neurons, the development of mitochondrial dysfunction and intracellular signaling pathways preventing cortical neuron death in L-homocysteine-induced excitotoxicity.

Supported by state program of IEPhB RAS.

NEW HSF1 INDUCERS AS PHARMACOLOGICAL TOOL FOR TREATMENT OF NEURODEGENERATIVE DISEASES

Lazarev V.F.[1], Ekimova I.V.[1,2], Margulis B.A.[1], Guzhova I.V.[1]

1 - Institute of Cytology of Russian Academy of Sciences, St.Petersburg, Russia
2 - M. Sechenov Institute of Evolutionary Physiology and Biochemistry of Russian Academy of Sciences, St. Petersburg, Russia

irina.guzh@gmail.com

Molecular chaperones whose expression is regulated by master HSF1 transcription factor, are known to provide the correct folding of cellular proteins as well as to exert cytoprotective and antiapoptotic activity. A therapeutic approach based on chemically induced chaperones expression may become a promising method to treat neurodegenerative disorders such as Parkinson disease or post-traumatic complications. The results of the studies performed on rodent models of above pathologies using chemically distinct HSF1 activators will be presented in relation to the prospect of their application in therapy of variety of proteotoxic diseases and in correcting aging-related factors.

OBTAINING AND DIRECTED DIFFERENTIATION INTO ASTROGLIAL AND NEURONAL DERIVATIVES OF IPSCS FROM PARKINSON'S DISEASE PATIENTS ASSOCIATED WITH THE GLUD2 MUTATION

Sorogina D.A.[1,2], Grigor'eva E.V.[2], Pavlova S.V.[2], Drozdova E.S.[1,2], Medvedev S.P.[2], Zakian S.M.[2]

1 - Novosibirsk State University, Novosibirsk, Russia
2 - The Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

d.sorogina@g.nsu.ru

Patient-specific cell models based on induced pluripotent stem cells (iPSCs) make it possible to study the mechanisms of various hereditary diseases development without surgical intervention in the human body. This is particularly useful for studying neurodegenerative diseases, including Parkinson's disease (PD).

The hereditary form of PD can be caused by mutations in various genes, one of which is the *GLUD2* gene. This gene is located on the X-chromosome and has no introns. *GLUD2* encodes the mitochondrial enzyme glutamate dehydrogenase 2, which involved in the oxidation of glutamate to α-ketoglutarate. *GLUD2* is expressed only in Sertoli cells, astrocytes, and neurons.

The T1492G mutation in the *GLUD2* gene leads to the replacement of Ser445Ala in the enzyme, which leads to an increase in its activity. Due to the increased degree of enzyme activity, dopaminergic neurons are disrupted, which leads to the development of PD. Males hemizygous for this mutation have an early onset of the disease. Heterozygous women are mosaics, so they get sick much later than men.

By reprogramming mononuclear cells, we obtained iPSC lines of two heterosexual patients with the T1492G mutation in the *GLUD2* gene. The cells were characterized by immunofluorescence staining and real-time PCR for pluripotency markers, as well as immunofluorescence staining of three germ layers obtained by spontaneous differentiation. These analyzes confirmed the pluripotency of iPSC lines.

After directed differentiation, astroglial and neuronal derivatives of iPSCs were obtained. These derivatives were characterized by immunofluorescent staining and quantitative PCR for specific markers of astrocytes and dopaminergic neurons.

The resulting cellular platform based on neural cells will be used to study the molecular genetic mechanisms of PD caused by a mutation in the *GLUD2* gene, as well as to test potential drugs.

The study was supported by the RSF grant No. 19-75-20063.

OPTOGENETIC ACTIVATION OF ASTROCYTES IN HIPPOCAMPUS RESTORES LTP FORMATION IN ALZHEIMER'S DISEASE MICE MODEL

Gerasimov E.[1], Erofeev A.[1], Borodinova A.[2], Bolshakova A.[1], Balaban P.[1,2], Bezprozvanny I.[1,3], Vlasova O.[1]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Cellular Neurobiology of Learning Lab, Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Science, Moscow, Russia
3 - Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, United States

evgeniigerasimov1997@gmail.com

Astrocytes are one of three types of nerve cells that are able to control neuronal activity by means of the gliotranmitters release and calcium level changes. Possible ways to control activity of them is a method of optogenetic. It gives researchers an opportunity to target control different type of cells activity, including astroglial cells. Previously was shown (Gerasimov et al., 2021), that metabotropic opsins (Opto-a1AR) are preferred for an astrocytic activation leading to enhancement of neuronal activity.

For understanding, whether optogenetic stimulation of metabotropic Opto-a1AR expressed in astrocytes could affect long-term potentiation changes, LTP experiments were conducted on acute brain hippocampal slices of WT and 5xFAD (model with genetic Alzheimer's disease) in age of 6 months after injection of AAV-Opto-a1AR-EYFP. Light parameters for activation of optoconstuct equaled 5 min of 100 ms pulses with interval of 1 sec and were determined in the previous work. Mean value of slope for last 5 min of recording were estimated. In WT group the percentage of mean slope value significantly increased after optogenetic stimulation: 162.8±7.0 (n=7) compared with 193,7±23,4 (n=4), p=0,0436, Student's t-test. In 5xFAD group the percentage of this values also significantly increased: 116.3 ±17.1 (n=8) vs 156.4±10.2 (n=4), $p<0.0001$, Student's t-test. Also, no significant changes were observed between WT group and 5xFAD group after optogenetic activation of Opto-a1AR: 162.8±7.0 (n=7) vs 156.4±10.2 (n=4), p=0.4742.

In this study was determined that impulse mode with T = 1 s, t = 100 ms parameters had an effect on formation of long term plasticity and leads to strengthen of synaptic transmission in Alzheimer's disease mice model. In the following research obtained data will be implemented in behavioral tests for comparison 5xFAD model mice with *in vivo* optogenetic stimulation and WT ones.

The work was supported by the Russian Science Foundation grant no. 20-65-46004.

OPTOGENETIC INHIBITION OF DRN SEROTONINERGIC NEURONS ABOLISHES THE ANTIDEPRESSANT EFFECT OF KETAMINE

Drozd U.S.[1,2], Lanshakov D.A.[1,2], Dygalo N.N.[1,2]

1 - Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia
2 - Novosibirsk State University, Novosibirsk, Russia

drozdnsu@gmail.com

Depression is a multifactorial disorder associated with the dysfunction of the brain's neurotransmitter systems, including serotonergic (5-HT) signaling, which is mediated by neurons mostly located in the dorsal raphe nucleus (DRN). Ketamine can produce a rapid antidepressant action even in patients considered treatment-resistant, but the mechanisms of its action are not fully understood. To clarify the possible role of 5-HT DRN neuronal activity in the drug effect, an antidepressant-like response to a subanesthetic dose of ketamine was evaluated in the tail suspension test (TST) in rats under optogenetic inhibition of 5-HT DRN neurons. For this, lentiviral vectors expressing the proton pump archaerhodopsin-3 (Arch) fused with the yellow fluorescent protein under the control of the TPH2 promoter were injected into DRN. A vector expressing fluorescent protein Venus was used as a control. The vector's expression was confirmed by immunohistochemical detection of the fluorescent proteins. Expression of c-Fos was used as a marker of neuronal activity and was evaluated by immunohistochemistry 1 hour after TST. Illumination of DRN with the green light inhibited the activity of neurons expressing Arch during TST. One hour after TST, the number of c-Fos expressing serotonergic cells in DRN was increased in ketamine-treated control rats, but if 5-HT neurons were optogenetically inhibited, a ketamine-induced increase in c-Fos expression was not observed. Ketamine reduced depressive-like behavior in animals with functionally active 5-HT neurons, but optogenetic inhibition of these neurons abolished the antidepressant effect of ketamine in TST and even increased the time of immobility. These data emphasize the key role of 5-HT neuronal activity in the rapid antidepressant effect of ketamine and could be necessary for the development of the next generation of fast-acting and more effective antidepressants.

Supported by FWNR-2022-0002.

PRENATAL HYPOXIA AS A FACTOR OF PREMATURE AGING OF THE BRAIN: GLUTAMATERGIC AND GLUCOCORTICOID-MEDIATED MECHANISMS

Tyulkova E.[1], Stratilov V.[1], Vetrovoy O.[1,2]

1 - Pavlov Institute of Physiology Russian Academy of Sciences, St. Petersburg, Russia
2 - Saint Petersburg State University, St. Petersburg, Russia

etyulkova@yandex.ru

Numerous physiological and clinical data show that intrauterine hypoxia is one of the main factors that can adversely affect fetal development. Violations of fetal development due to hypoxic stress lead to the formation of pathologies of postnatal development. This study was aimed at analyzing the characteristics of the glucocorticoid and glutamatergic systems and behavior during early, adult postnatal ontogeny and in aged rats subjected to prenatal hypoxia (PH) in the critical period of hippocampal development (prenatal days 14-16). We have shown an age-related decrease in the amount of glutamate in the hippocampus of PH rats, which is accompanied by a decrease in the number of neuronal cells in the CA1 field of the hippocampus, as well as a decline of spatial memory in the Morris water maze. A gradual decrease in the amount of glutamate is associated with insufficient expression of glutamate metabolism genes and is inversely correlated with a compensatory increase in the levels of mGluR1. We have shown that in the hippocampus of adult PH rats, in contrast to control animals, in response to a session of severe hypoxia used as an excitotoxic model, there is no increase in the generation of lipid peroxidation products. This indicates that the excessive activity of the receptor part of the glutamate system in PH animals does not balance the deficiency of glutamate. Meanwhile, the maternal stress response to hypoxia mediates a stable violation of the sensitivity of the fetal hippocampus to glucocorticoids, which further determines the central and peripheral dysfunctions of the glucocorticoid system. The results of the study demonstrate significant contribution of dysfunction of the glutamatergic and the glucocorticoid systems to early aging, which manifests itself in the age-related decline of the cognitive functions of PH rats, early neuronal loss, and early mortality.

PRIMUM NON NOCERE OR HOW TO CREATE EFFECTIVE AND HARMLESS FOR CHRONIC NEUROPATHIC PAIN THERAPY

Shestakova N.N.

Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St. Petersburg, Russia

n_shestakova@list.ru

The analysis of the strategies developed and implemented by us for creating an effective and safe therapy for patients with chronic neuropathic pain syndrome, based on the results of a comprehensive chemical and pharmacological approach, including molecular computer modeling, electrophysiological and biochemical methods, and clinical trials. The creation of adjuvant analgesic therapy without increasing the number of prescribed drugs. Indirect actions on the glutamatergic system provides effective but safe neuropathic pain relief. Rationale for the creation of new classes of analgesics.

1. Stepanenko, Yulia D et al. “Tricyclic antidepressant structure-related alterations in calcium-dependent inhibition and open-channel block of NMDA receptors”. Front. Pharmacol. – Neuropharmacology. 2022 Feb 14; doi: 10.3389/fphar.2021.815368
2. Belinskaia, Daria A et al. “Psychotropic Drugs for the Management of Chronic Pain and Itch.” Pharmaceuticals (Basel, Switzerland) vol. 12,2 99. 24 Jun. 2019, doi:10.3390/ph12020099
3. Uniyal, Ankit et al. “Tozasertib Attenuates Neuropathic Pain by Interfering with Aurora Kinase and KIF11 Mediated Nociception.” ACS chemical neuroscience vol. 12,11 (2021): 1948-1960. doi:10.1021/acschemneuro.1c00043
4. Stepanenko, Yulia D et al. “Dual action of amitriptyline on NMDA receptors: enhancement of Ca-dependent desensitization and trapping channel block.” Scientific reports vol. 9,1 19454. 19 Dec. 2019, doi:10.1038/s41598-019-56072-z

These studies were carried out within the State assignment AAAA-A18-118012290427-7 and supported by RFBR grant 20-515-18008.

PAIRED OPTOGENETIC STIMULATION OF MOUSE VISUAL CORTEX NEURONS CAN CHANGE THEIR RESPONSE PROPERTIES

Smirnov I.V., Malyshev A.Yu.

Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia

ivan.vas.smirnov@gmail.com

Synaptic plasticity is an ability of synapses to change their strength over time. According to the one of the most common paradigm of modern neurobiology, synaptic plasticity can play an important role in processes of memory formation. However, most studies of cellular and molecular mechanisms of synaptic plasticity are performed on simplified in vitro models, which does not provide a complete picture of the role of these mechanisms in the functioning of the brain. In our study, we investigate the role of synaptic plasticity in modifications of cellular responses in the mouse primary visual cortex *in vivo*.

To assess the state of the synaptic inputs of the studied cell, we used visual stimulation, presenting the moving vertical and horizontal bars to the animal. To characterize the neuronal responses, we used orientation and direction selectivity indices.

In our study, we paired non-optimally oriented visual stimuli with optogenetics stimulation of individual neurons of the layer V of the primary visual cortex in transgenic mice expressing ChR2 under the Thy promoter. Neurons were recorded using juxtracellular technique. Optogenetic stimulation of the recorded neuron was performed via optical fiber placed in the recording microelectrode and connected to the blue LED. During the plasticity induction, 100 paired stimulations were performed. Paired stimulation caused a change in the optimal orientation of the studied neurons, which was expressed in an increase in the response to the reinforced orientation and lasted at least one hour after pairing. In addition, we found that this paired stimulation can change directional selectivity of the stimulated neurons.

This study was supported by the Russian Science Foundation (grant #20-15-00398).

PATIENT-SPECIFIC MODELS OF PARKINSON'S DISEASE AS A PLATFORM FOR STUDYING ABERRANT CALCIUM SIGNALING

Grekhnev D.A., Novikova Yu.V., Kruchinina A.A., Vigont V.A., Kaznacheyeva E.V.

Institute of cytology RAS, St Petersburg, Russia

dima.grehnyov@yandex.ru

Impaired calcium signaling has been noted in various animal and cellular models of neurodegenerative disorders, including Parkinson's disease (PD). Patient-specific models allow the most accurate reproduction of the pathological phenotype of the patients and open new horizons in studying neurodegenerative pathologies. Directed differentiation of stem cells makes it possible to study molecular mechanisms underlying pathology on various types of cells, including the most vulnerable for this particular disorder. Our research project was focused on the use of novel patient-specific models of PD to study the alterations of calcium signaling and to identify molecular targets for promising antiparkinsonian drugs. Here we used iPSC-derived dopaminergic neurons as the most vulnerable cells in PD. More than 95% of neurons expressed a specific marker of dopaminergic neurons, tyrosine hydroxylase, and were able to generate action potentials, which indicated the functional maturity of the neurons. Using a patch-clamp technique we showed an increased store-operated calcium entry (SOCE) in dopaminergic neurons with G2019S mutation in the *LRRK2* gene. For detail studying the effect of G2019S mutation in *LRRK2* on SOCE, we used an isogenic system with the introduction of this mutation in *LRRK2* (into the second allele) and with the correction of this mutation or with the knockout of the mutant allele. For cell lines carrying the G2019S mutation in one or both alleles, we showed an increase in SOCE. Moreover, the SOCE level did not vary for homo- and heterozygotes states of the mutant gene. At the same time, dopaminergic neurons, both with the correction of this mutation and with the knockout of the mutant allele, had no changes in the amplitude of SOCE compared with the neurons differentiated from the healthy donors. The study was supported by a grant from the Ministry of Science and Higher Education of Russia (Agreement No. 075-15-2021-1075, 28.09.2021).

PHOTOPHARMACOLOGICAL AND OPTOSENSORIC ANALYSIS OF NEURONAL FUNCTIONS

Bregestovski P.[1,2,3], Ponomareva D.[1,2,3], Petukhova E.[2,3]

1 - Institut de Neurosciences des Systèmes, Aix-Marseille University, INSERM, INS, 13005 Marseille, France
2 - Institute of Neurosciences, Kazan State Medical University, Kazan, Russia
3 - Department of Normal Physiology, Kazan State Medical University, Kazan, Russia

pbreges@gmail.com

The photopharmacology and optosensorics represent novel tools for modulating and analysis of neuronal activity in various experimental models. The photopharmacology is aimed at creating and functional analysis of new photocontrolled compounds regulating the inhibitory activity of the brain. The optosensorics is aimed at creating models for non-invasive monitoring of intracellular ion concentrations using genetically encoded biosensors constructed on the basis of fluorescent proteins.

Within the framework of photopharmacology, we analyzed the specificity of the action of the compound Glyght which was recently demonstrated to modulate activity of glycine receptors in photocontrolled manner. Glycine receptors belong to the family of Cys-loop transmembrane proteins and provide inhibition in the spinal cord, retina, and in some parts of the brain. To find out the effects of the Glyght on inhibitory GABA receptors, we performed experiments on hippocampal neurons of mouse brain slices analyzing synaptic currents. It has been shown that Glyght does not directly modulate the activity of GABA receptors. Our observations prove the high selectivity of the action of a new photochrome on glycinergic synapses in the mammalian nervous system. Within the framework of another area, optosensorics, we analyzed intracellular concentrations of chloride (Cl-) and hydrogen (H+) ions in neurons of brain slices and presynaptic neuromuscular junctions of transgenic mice expressing a fluorescent probe, ClopHensor, which allows simultaneous and non-invasive registration of these ions concentrations. The effectiveness and reliability of ClopHensor has been tested under various experimental conditions. We analyzed changes in ions during depolarization caused by an increase in external potassium ions, as well as high-frequency synaptic stimulation of Schaffer's collaterals of hippocampal slices. The results illuminate the various ways of regulation of Cl- and H+ equilibrium in neurons and demonstrate that different transport systems are involved in maintaining the homeostasis of these ions.

Supported by the RSF grant: 18-15-00313.

PI-HELICAL ELEMENTS IN THE INNER HELICES OF SODIUM, POTASSIUM AND TRP CHANNELS

Tikhonov D.B.[1], Zhorov B.S.[1,2]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia
2 - McMaster University, Hamilton, Canada

denistikhonov2002@yahoo.com

The rapidly increasing number of experimental 3D structures of sodium, calcium and TRP channels, which belong to the super-family of P-loop channels, revealed an interesting previously unrecognized feature. In some structures S6 transmembrane segments, which line the inner pore and form the activation gate, are not entirely alpha-helical, but contain a pi-helical element. Since a pi-helix has an additional residue per helical turn, it causes a ~90 degrees rotation of the helical C-part. This drastically changes patterns of the helix contacts with other segments and the pore-bound ligands. In some cases, appearance/disappearance of pi-helical elements is accompanied by significant changes of the pore lumen dimensions suggesting that the pi-helical elements may function as gating hinges in the channels, which lack the glycine hinge present in potassium channels. Indeed, structures of the same channels in different states often differ by the presence or absence of pi-helical elements. Moreover, there are examples when pi-helical elements appear or disappear depending on the drug binding in the pore. The latter phenomenon should be taken into consideration to rationalize intriguing peculiarities of action of sodium channels activators, e.g., batrachotoxin and veratridine and their channel-blocking derivatives, as well as the action of dihydropyridine agonists and antagonists on calcium channels. The AlphaFold2 neural network also predicts pi-helical elements in S6 helices of sodium, calcium and TRP channels. However, we do not see a rule of appearance of the pi-helical elements and their patterns usually do not coincide between the predicted and experimental structures. Likely, structures with and without pi-helical elements have close energies and their appearance depends on various factors. These new features of P-loop channels, which are of surmount importance in physiology, pathophysiology, pharmacology and toxicology, require further studies.

POSITIVE TRPC6 MODULATOR TREATS SYNAPSE DEFICIENCY IN ALZHEIMER'S DISEASE HIPPOCAMPAL NEURONS

Zernov N.I.[1], Bezprozvanny I.B.[1,2], Popugaeva E.A.[1]

1 - Peter the Great St Petersburg Polytechnic University, Laboratory of Molecular Neurodegeneration, St. Petersburg, Russia
2 - UT Southwestern Medical Center, Department of Physiology, Dallas, USA

quakenbush97@gmail.com

Alzheimer's disease (AD) is an incurable form of dementia. The number of individuals worldwide with AD is growing at a rapid rate. New treatments are urgently needed.

AD is characterized by synaptic dysfunction that leads to slowly progressing memory loss. We have recently discovered a novel transient receptor potential canonical 6 (TRPC6)–mediated intracellular signaling pathway that regulates the stability of dendritic spines and plays a role in memory formation [1]. We assume that the positive modulators of TRPC6 may stabilize synaptic contacts, thereby preventing memory loss.

We have previously shown that TRPC6 agonists exert beneficial effects in models of AD and may serve as therapeutic agents for AD [1, 2]. In current study, we demonstrated that another TRPC6 modulator, derivative of benzopyran, shows neuroprotective effects. We have observed that treatment with 1 uM or 100 nM of benzopyran derivative recovers the percentage of mushroom spines in the Aβ42-treated group. We have also observed that 100 nM benzopyran derivative completely rescued the long-term potentiation deficit in slices from 5xFAD mice. Besides, it is stable in mouse plasma for 4 hours and efficiently crosses the blood–brain barrier. Experimental evidence suggests that benzopyran derivative is a potential lead molecule to prevent synapse deficiency in AD hippocampal neurons.

The study was supported by Russian Science Foundation (Project No 20-75-10026).

1. Zhang, H, Sun, S., Wu, L., Pchitskaya, E., Zakharova, O., Fon Tacer, K., Bezprozvanny, I. (2016). Store-Operated Calcium Channel Complex in Postsynaptic Spines: A New Therapeutic Target for Alzheimer's Disease Treatment. Journal of Neuroscience, 36(47), 11837–11850.
2. Popugaeva, E., Chernyuk, D., Zhang, H., Postnikova, T. Y., Pats, K., Fedorova, E., Poroikov, V., Zaitsev, A. V., Bezprozvanny, I. (2019). Derivatives of Piperazines as Potential Therapeutic Agents for Alzheimer's Disease. Molecular Pharmacology, 95(4), 337–348.

POSITIVE ALLOSTERIC MODULATORS OF SK CHANNELS NORMALIZE FIRING ACTIVITY OF CEREBELLAR PCS FROM SCA2-58Q TRANSGENIC MICE

Egorova P.A.[1], Bezprozvanny I.B.[1,2]

1 - Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - University of Texas Southwestern Medical Center, Dallas, TX, USA

bio_polya@mail.ru

The accurate firing of the cerebellar Purkinje cells (PCs) is crucial for the proper cerebellar functioning. In our aging SCA2-58Q transgenic mice we previously observed the gradually increasing portion of PCs with highly irregular bursting activity. Consistent with our preceding results, in the current study we also observed that most of PCs from 7-8 months old WT mice (97 ± 2%, n = 56 PCs) were firing tonically, whereas much less PCs from SCA2 mice of the same age were having stable firing rates (75 ± 4%, n = 119 PCs, **p < 0.01). Thus, every fourth SCA2-58Q PC cell exhibited bursting activity patterns. We believe that highly irregular bursting activity reflects the consequences of the ionic imbalance observed in SCA2 PCs leading to the motor decline in SCA2 mice. Thus, the compounds that can convert bursting activity into the tonic mode may have the potential therapeutic effect for SCA2 and other ataxias.

To investigate if positive allosteric modulators of SK channels can rescue the abnormal firing of SCA2-58Q PCs, we performed the series of experiments with acute cerebellar slices from 7-8-month-old SCA2-58Q mice in the presence of the pan-SK channel modulator CHZ (chlorzoxazone) and the selective SK2 modulators 2o and 2q developed by the group of Miao Zhang. Application of 50 uM CHZ converted bursting SCA2-58Q PC to tonic firing pattern, and also significantly decreased the firing frequency of the cell and improved the regularity of PC activity. Application of 10 uM 2o converted bursting SCA2-58Q PC to tonic firing pattern too, and significantly decreased the firing frequency of the cell and improved the regularity of PC activity as well. Application of 10 uM 2q converted bursting SCA2-58Q PC to tonic firing pattern too, and significantly decreased the firing frequency of the cell and improved the regularity of PC activity as well. However, in 8 minutes after 2q application, the activity of the cell switched to the silent mode. This may be explained by the higher potency of 2q compared to 2o. Thus, with a similar concentration of tested drugs, 2q compound causes more dramatic effect than 2o compound and limits the firing activity of PCs. All together, these experiments suggested that activation of SK2 channels restores tonic firing of PC cells in aging SCA2 mice. Thus, the application of positive allosteric modulators of SK channels results in a reliable increase in regularity of SCA2-58Q PC firing pattern and hereby may have a potential therapeutic effect for SCA treatment.

POSSIBLE NO-ERGIC REGULATION OF RETINAL LIGHT SENSITIVITY IN LYMNAEA STAGNALIS

Saphonov M.V., Dominova I.N., Zhukov V.V.

Immanuel Kant Baltic Federal University, Kaliningrad, Russia

mikhailsafonov96@mail.ru

The molecular processes underlying light sensitivity and its regulation in gastropods remain to be poorly understood. The retina of the camera-like eye of the *Lymnaea stagnalis* contains numerous microvillar photoreceptors that provide high light sensitivity and spatial resolution of vision. The retina itself receives efferent innervation, partly FMRF-amide-ergic one. Based on evidence that FMRF-amide modulates the NO formation (Röszer et al., 2004) and immunochemical detection of NO synthase (NOS) in the retina of *Helix pomatia* (Huang et al, 1997), we undertook a search for NO-ergic mechanism in the eye of *L. stagnalis*. We looked for transcription of NOS genes in the retina and assessed the effect of NO on electroretinogram (ERG).

To check the presence of NOS transcripts in the snail brain and eye, we extracted RNA and carried out qPCR (primers were designed by Korneev et al., 2005). The transcription levels (TLs) of 2 *NOS* gene isoforms and *antiNOS* intergenic region were normalized to the *Gapdh* and calculated using the 2ddCt method. Statistical analysis was performed using the one-way ANOVA with post-hoc Tukey test ($p < 0.05$).

So, we determined the transcription of both *NOS* isoforms in the snail brain and eye, as well as, *antiNOS* in these tissues. The *NOS1* and *NOS2* TLs were significantly upregulated in the eye compared to the brain. But, the *antiNOS* TLs were about the same in both samples.

In electrophysiological studies, a reversible inhibitory effect of the nitric oxide donor SNAP 0.1 mM on the ERG of an isolated snail's eye was established. This effect developed within 30 minutes after the drug application and declined several hours after returning to saline. Although the results obtained are preliminary, they suggest that NO is involved in the modulation of retinal processes.

M.S. was granted by IKBFU to support the research work of graduate students.

POSTTRAUMATIC AND IDIOPATHIC SPIKE-WAVE DISCHARGES IN RATS: DIFFERENTIATION BY MORPHOLOGY AND SUBCORTICAL STRUCTURES INVOLVEMENT

Salyp O.Yu.[1], Komoltsev I.G.[1,2], Volkova A.A.[1], Bashkatova D.A.[1], Shirobokova N.I.[1], Frankevich S.O.[1,2], Shalneva D.[1], Kostyunina O.V.[1], Chizhova O.[1], Kostukov P.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

olgha.list@inbox.ru

Introduction: Post-traumatic spike-wave discharges (SWDs) disrupt brain activity on cellular and molecular levels and may be associated with epileptogenic mechanisms. Previously we have shown an increased SWD occurrence in the neocortex 7 days after TBI in rats. We hypothesised that formation of SWDs involves the cortico-thalamo-cortical neuronal network.

Materials and methods: The experiments were performed on 12 adult male Sprague-Dawley rats divided into two groups: sham-operated (n=5) and TBI (n=7). Eight nichrome depth electrodes were implanted into the sensory cortex, dorsal and ventral hippocampal dentate gyrus (bilaterally) and thalamic right ventro-posterior-lateral nucleus (unilaterally). On day 7 after electrode implantation lateral fluid percussion brain injury was performed (2.5 atm). Local field potentials (LFP) were recorded 24 h/day during 7 days before and 7 days after TBI.

Results: The average number of SWDs per day increased after TBI (2 SWD/day before, 150 SWD/day after). After TBI, SWDs were recorded simultaneously in the neocortex and thalamus, though this phenomenon was not observed before TBI. Post-traumatic discharges were different from idiopathic ones according to several characteristics, in particular, higher proportions of bilateral spreading, spike-waveform and thalamus involvement ($p<0.001$). Presence of SWDs in the thalamus was associated with their bilateral spreading in the cortex, spike-waveform of discharges and the presence in the hippocampus ($p<0.001$). Based on SWD parameters we can determine thalamic involvement and etiology with an accuracy of 82% and 75%, respctively.

Conclusion: Our results support the hypothesis that formation of post-traumatic SWDs involves a cortico-thalamo-cortical neuronal network. The results can be used for further search of molecular mechanisms associated with the occurrence of epileptiform activity and epileptogenesis.

Supported by RSF, grant № 22-25-00713

PRENATAL HYPOXIA ACTIVATES THE MECHANISMS OF NICOTINE ADDICTION

Stratilov V.A.[1], Vetrovoy O.V.[1,2], Tyulkova E.A.[1]

1 - Pavlov Institute of Physiology RAS, Saint-Petersburg, Russia
2 - Saint-Petersburg State University, Saint-Petersburg, Russia

stratilov.v@infran.ru

The role of damaging factors in prenatal period as basis of drug addiction in offspring is of great interest. Our investigation aims to study the effects and possible mechanisms of prenatal hypoxia (PSH) on the vulnerability to nicotine addiction in adult rats. In PSH rats, we have revealed an increased tendency to nicotine consumption in two-bottle choice test. After two weeks of chronic treatment with nicotine (Alzet osmotic minipump, 9 mg/kg per day), we assessed the sights of withdrawal in the conditioned place aversion test with mecamylamine in dose 1 mg/kg (antagonist of nicotinic acetylcholine receptors, nAChR). We have shown that the mecamylamine-precipitated withdrawal aversion is stronger in PSH group than in the Control group. So, PSH might be considered as predisposing factor. PSH rats also demonstrated an increase in the proportion of phosphorylated DARPP-32 protein (known as the relay for dopamine and glutamate signaling) at 34 threonine residue (pThr34DARPP-32) in relation to its total amount in the nucleus accumbens of striatum (NAc), meanwhile changes in both content of dopamine in the mesolimbic pathway and the first type of dopamine receptors (DAR1) in NAc were absent. Probably, increasing the DARPP-32 phosphorylation rate in adult PSH rats is a consequence of overglutamate stimulation of the ventral tegmental area (VTA) dopaminergic (DA) neurons, in response to nicotine activation of presynaptic nAChR. This hypothesis is supported by observed increase in VGluT2 positive terminals to Nurr1 positive neuronal bodies in VTA.

Thus, an altered glutamate phenotype might play significant role in the development of PSH-related nicotine addiction.

Supported by RFBR grant № 19-315-90003.

PROPAGATION OF CORTICAL SPREADING DEPRESSION IN SOMATOSENSORY CORTEX OF RATS WITH PRENATAL HYPERHOMOCYSTEINEMIA

Gerasimova E.[1,2], Enikeev D.R.[1], Sitdikova G.F.[1]

1 - Kazan Federal University, Kazan, Russia
2 - Sirius University of Science and Technology, Sochi, Russia

gerasimova.el.2011@yandex.ru

Homocysteine (Hcy) is an endogenous redox active amino acid that is produced in the metabolic cycle of methionine by a transmethylation reaction. Recent meta-analysis suggested a possible link between elevated serum Hcy level and migraine, especially migraine with aura. Cortical spreading depression (CSD) is a slowly propagating wave of transient neuronal and glial depolarization evoked by elevation of extracellular K+ concentration and considered as an electrophysiological correlate of aura. In this work, we evaluated CSD in the somatosensory cortex of rats with prenatal hyperhomocysteinemia.

Experiments were performed on Wistar male rats (P40-60). Rats with prenatal hyperhomocysteinemia (Hcy group) were born from females which received daily methionine (7.7 g/kg food) starting three weeks prior and during pregnancy. CSD was recorded from the primary somatosensory cortex, S1, using 16-site linear silicon probes (Neuronexus technologies, MI) in vivo. CSD was induced by KCl (1M) and was recorded during 2 hours. 2,3,5-triphenyltetrazolium chloride (TTC) was used for identification of damaged tissue after electrophysiological experiment.

Topical two hours KCl application induced CSD generated in the supragranular layers (L2/3), and then spreading to the granular (L4) and infragranular layers (L5/6). In control group we observed from 1 to 34 CSD, whereas in Hcy group – 9 - 43 CSD. The amplitude of CSD was lower and duration of CSD was longer in rats from HCY group. Recurrent CSDs in the control group mainly generated in the supra granular and granular layers whereas in Hcy group CSD propagated mainly in granular and supragranular layers. Subsequent staining of brain slices with TTC revealed the necrotic area of somatosensory cortex in rats from hHCY group.

Our data suggests that recurrent CSDs in rats with hyperhomocysteinemia induce metabolic alteration with subsequent neuronal damage which suggests lower resistance of neuronal tissues to depolarization.

This work was supported by the Russian Science Foundation (grant number 20-15-00100)

PROTECTIVE AND ANTIOXIDANT EFFECTS OF INSULIN AND α-TOCOPHEROL ON BRAIN NEURONS AND THEIR MOLECULAR MECHANISMS (IN VITRO AND IN VIVO)

Zakharova I.O., Bayunova L.V., Zorina I.I., Sokolova T.V., Shpakov A.O., Avrova N.F.

Laboratory of Molecular Endocrinology and Neurochemistry, Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

avrova@iephb.ru

Clinical trials show that intranasal insulin (I-I) is a promising drug for the treatment of neurodegenerative and other brain diseases. But long-term administration of high-dose I-I may result in the development of central insulin resistance. It seems of importance to reveal compouns that can enhance the neuroprotective effect of insulin. These substances may include alpha-tocopherol (α-T), the main component of vitamin E. Our aim was to study the protective and antioxidant effects of insulin and α-T *in vitro* and *in vivo*. The ability of insulin and α-T to enhance the protective effect of each other on cultured neurons in oxidative stress and the efficiency of co-administration of I-I and α-T to normalize the metabolic disturbances caused by hyperactivation of free radical reactions in brain cortex of rats with two-vessel forebrain ischemia/reperfusion injury were investigated. Immunoblotting, flow cytometry, colorimetric and fluorometric techniques were used in the study. For the first time α-T was shown to enhance markedly the neuroprotective, antiapoptotic and antioxidant effects of insulin in brain cortical neurons in oxidative stress, these effects being additive. At the late stages of oxidative stress, the combination of insulin and α-T increased the Akt kinase, inhibited the GSK 3-beta and normalized the ERK1/2 activity in cortical neurons to a greater extent than each protector alone. Two-vessel forebrain ischemia and subsequent reperfusion increased lipid peroxidation product content and caused oxidative inactivation of Na^+,K^+-ATPase in the rat brain cortex. Co-administration of I-I (0.25 IU/rat) and α-T (orally, 50 mg/kg) to ischemic rats led to a more pronounced normalization of the Schiff bases, conjugated dienes and trienes levels and the Na^+, K^+-ATPase activity in the brain cortex than administration of one of them. Further studies will show whether the combined use of I-I and α-T is able to prevent neuropathy in ischemia and other brain injury.

Supported by funds from the Ministry of Science and Higher Education of Russian Federation (NCME “Pavlovsky Center” No 075-15-2020-916).

QUANTITATIVE ANALYSIS OF MINISCOPE NEURONAL ACTIVITY DATA

Gerasimov E.[1], Pchitskaya E.[1], Mitenev A.[1], Vasiliev P.[1], Erofeev A.[1], Chukanov V.[1], Vlasova O.[1], Bezprozvanny I.[1,2]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, USA

evgeniigerasimov1997@gmail.com

Visualization of neuronal activity *in vivo* is an urgent task in modern neurobiology. It could give large amount of information about changes in neuronal connectivity in neurodegenerative diseases and probably might show functional impairments at early stages of them. An original approach that allowed obtaining neuronal activity *in vivo* in non-fixed animal Miniscope.

In current research, injection of gCamp6 virus were performed in hippocampus (AP -2.1, ML +2.1, DV -1,8) of 5 month FVB mice and after 3 weeks GRIN-lens was attached above area of interest. Changes in calcium levels was recorded by Miniscope v2 at mouse's home cage.

Miniscope gives researchers an opportunity to determine different parameters of neuronal activation of various brain regions with subsequent analysis, however, it is complex task. To overcome this, a novel software package, performed on Python, was developed for high-level metrics evaluation from processed data. It allows to calculate different statistics for neuronal activation such as: burst rate (amount of single neuron activation in period of time), network spike rate (quantity of active neurons in period of time), network spike peak (highest number of simultaneously active neurons) and mean correlation (Pearson coefficient). Determination of the active state of a neuron is performed based on derivatives of input signals after their preprocessing. For this purpose, a threshold value is calculated based on statistics. A neuron is in an active state if the derivative of its signal exceeds the threshold value.

This tool will provide possibility of high-level processing of miniscope imaged neuronal data. In our future investigations, this quantitative metrics will find applications in comparisons of hippocampal neuronal network activity in wild type mice and mice models of Alzheimer's disease in behavioral tests.

The work was supported by the strategic academic leadership program "Priority 2030" of the Russian Federation (Agreement 75-15-2021-1333 30.09.2021 to SPbPU).

ROS GENERATION IN THE BRAIN IN COURSE OF THE ROTENONE MODEL OF PARKINSON DISEASE

Ayvazyan N.M., Kirakosyan G.R., Zaqaryan N.A., Ghazaryan D., Tadevosyan H., Chavushyan-Papyan V.A.

Orbeli Institute of Physiology of NAS RA, Yerevan, Armenia

taipan@ysu.am

Parkinson disease (PD) is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) and the presence of intracytoplasmatic inclusions known as Lewy bodies. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. A clinically-related animal model of Parkinson's disease (PD) may enable the elucidation of the etiology of the disease and assist the development of medications. We investigated the balance of free radicals in brain different fractions (striatum, brain stem, neocortex, cerebellum & spinal chord) and some tissues (liver, heart, timus) of rats in course of *in vivo* processing by *rotenone,* a clinically-related animal model of Parkinson's disease (PD). Chemiluminescence (ChL) levels were examine in tissue assays on the 5^{th}, 10^{th} and 15^{th} days after stereotaxical (ST) infusion of small doses of the mitochondrial complex-I inhibitor, rotenone, into the right medial forebrain bandle area. The TBA-test was also performed to confirm the free radical expression. The activity of superoxide dismutase in isolated tissue fractions were detected by spectrophotometry. Five days after ST rotenone administration, chemiluminescence levels of tissue homogenates significantly decreased in all fractions except of striatum, while in 10^{th} and moreover in 15^{th} days of rotenone intoxication the level of ChL were elevated; lipid peroxidation also decreased in all fractions at5^{th} and 10^{th} days, but there were no significant balance changes in the 15^{th} day of treatment. On the contrary, the activity of superoxide dismutase was not shown any tendencies to change in all tissues, except of the neocortex and liver. In the latter's we observed the dramatically increased activity of the enzyme at the 10^{th} day of rotenone injections.

RADIATION-RESISTANT MICROFLUIDIC LAB-ON-CHIP SYSTEM FOR CULTURING NEURONS TO STYDY THE EFFECT OF MICROGRAVITY AND RADIATION CONDITIONS ON THE DEVELOPMENT OF NEURODEGENERATIVE DISEASES

Iakovlev A.P.[1], Marchenko O.M.[2], Shemukhin A.A.[2], Kolmogorov V.S.[1,2], Erofeev A.S.[1,2], Gorelkin V.S.[1], Korchev Y.E.[1,3,4]

1 - National University of Science and Technology «MISiS», Moscow, Russia
2 - Lomonosov Moscow State University, Moscow, Russia
3 - Department of Medicine, Imperial College London, London, UK
4 - D. Mendeleev University of Chemical Technology of Russia, Moscow, Russia

yakovlevap74@gmail.com

Parkinson's disease and multiple sclerosis are not developmental disorders and modeling these disease states in vitro is too complicated. More complex models containing multiple cell types, and fully mature cells are used to better represent the disease progression, allowing for a more suitable platform for development of potential therapies. Experiments in orbit on the International Space Station (ISS) demonstrated changes in the composition of the extracellular matrix, apoptosis, differentiation, and growth behavior of the cells. The effect of microgravity on human brain organoids is a promising area of scientific interests because they may help understand how neuronal systems adapt to microgravity. Moreover, they serve as a useful 3-dimensional human model system to understand the biological processes involved in neurological disease and to identify potential new treatment options. We have developed an active autonomous microfluidic system for cell cultivation under conditions of high background radiation and microgravity, which is capable of working aboard the ISS. Developed system corresponds to the 2U parameter (the volume occupied by the system does not exceed 20 cm3). The unique structure of the device allows cell cultivation for more than 30 days, while the flow generated by the system is in the range from 0 to 40 µl/hour The autonomy of the developed system allows it to be completely isolated from humans, which can significantly reduce the amount of work carried out on the ISS, thereby reducing the already costly space research. The developed system is a new step in the study of the development of human pathologies, as well as in testing new drugs on models of human organs.

We have developed this system within The Russian Science Foundation, grant number: № 19-79-30062.

REFERENCE GENE STABILITY WITHIN THE RAT BRAIN IN THE LITHIUM PILOCARPINE EPILEPSY MODEL: TIMING AND REGION SPECIFICITY

Schwarz A.P.[1], Kovalenko A.A.[1], Zakharova M.V.[1], Dyomina A.V.[1], Trofimov A.N.[2], Malygina D.A.[1], Zubareva O.E.[1], Zaitsev A.V.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia
2 - Institute of Experimental Medicine, St. Petersburg, Russia

aleksandr.pavlovich.schwarz@gmail.com

Reverse transcription followed by quantitative polymerase chain reaction (qRT-PCR) is a powerful and commonly used tool for gene expression analysis. It requires the right choice of stably expressed reference genes for accurate normalization. In this work, we aimed to select the optimal reference genes for qRT-PCR normalization within different rat brain areas at different stages of the Li-pilocarpine model of acquired epilepsy. We have tested the expression stability of nine housekeeping genes: *Actb, Gapdh, B2m*, *Rpl13a, Sdha, Ppia*, *Hprt1, Pgk1,*and *Ywhaz.* We have developed set of original multiplex qPCR assays allowing to analyse expression of aforementioned genes in 3 reactions. Designed assays demonstrate optimal efficiency and repeatability. Based on geometric averaging of ranks obtained by four common algorithms (geNorm, NormFinder, BestKeeper, Comparative Delta-Ct),we found that the stability of tested reference genes varied significantly between different brain regions after pilocarpine induced status epilepticus and depends on timing (3 days, 7 days in latent phase of the model, or 2 monts, i.e. chronic phase). *Pgk1* and *Ywhaz* were the most stable, while *B2m* demonstrate the lowest stability in the analysed brain areas. *Gapdh* expression was one of the most stable in the hippocampus, but it has low stability in the medial prefrontal and temporal cortical areas, and amygdala. High reference gene stability were detected in the medial prefrontal cortex, amygdala, and dorsal hippocampus, whereas in the ventral hippocampus and temporal cortex 4-5 of 9 analysed genes were unstably expressed and inappropriate for expression normalisation. Within the analysed brain regions, the stabilities of tested reference gene expression were lower 3 days (early latent phase of the model), and especially 2 months after pilocarpine induced seizures compared to 7 day post-seizure rats.Thus, the reference genes for RT-qPCR data normalisation in the rat Li-pilocarpine should be differentially selected based on specific brain area and time after the induction of epileptogenesis.

Supported by Russian Science Foundation, grant № 20-75-00127.

REMOTE COGNITIVE DISTURBANCES IN RATS AFTER LATERAL FLUID PERCUSSION BRAIN INJURY

Frankevich S.O.[1,2], Komoltsev I.G.[1,2], Volkova A.A.[1], Shirobokova N.I.[1], Salyp O.[1], Shalneva D.[1], Bashkatova D.[1], Kostyunina O.V.[1], Chizhova O.[1], Kostrukov P.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Department of Functional Biochemistry of Nervous System, Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

stepan.frankevich@yandex.ru

Introduction: Patients recovering after traumatic brain injury (TBI) are suffering from memory loss, impared spatial sense and other late-onset complications. As a part of a large study of remote effects of TBI and their mechanisms, we examined acute and remote behavioral changes in rats after a lateral fluid-percussion brain injury in Barnes maze test.

Methods: The experiment was performed on 51 Sprague-Dawley rats divided into a control group, TBI group and sham operation group. Rats from the TBI group received a lateral fluid-percussion brain injury, while the sham-operation group was subjected to respective trepanation. Experimental animals were subjected to a standard Barnes test to assess spatial memory in three series of tests: Barnes 1 (learning, before craniotomy), Barnes 2 (acute period, days 6-8 after TBI) and Barnes 3 (chronic period, 7 days long testing 3 months after TBI). On the 1st day of Barnes 3 test, the escape tunnel was moved for reversal learning.

Results: During the learning session all animals demonstrated steady decline of time to tunnel location, indicating spatial learning, with no differences between groups. There were no changes in behavior in the acute period of TBI. Three months after injury, rats in the TBI group moved a larger cumulative distance than rats in the sham group (Barnes 3, days 1, 2 and 6, $p<0.05$). Three months after TBI, rats from TBI group needed more time to escape maze in comparison with sham (Barnes 3, days 1 and 2, $p<0.05$) and control groups (Barnes 3, day 2, $p<0.05$). No significant changes in animal locomotor activity were observed.

Conclusion: Our results demonstrate impairments of spatial memory 3 months after TBI. Slow development of cognitive impairment may be caused by glucocorticoid-dependent hippocampal degeneration revealed in our previous studies.

Supported by RSF, grant №22-25-00713

ROLE OF MICROBIOTA IN GUT-BRAIN AXIS

Sobol C.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

peep9@yandex.ru

Functional interaction of the gastrointestinal tract (GI) and the central nervous system (CNS) is due to various relationships, which includes autonomic and enteral nervous systems as well as the immune and neuroendocrine systems. The microbiota of the macroorganism plays the central role in this interaction. Microbiota produces hundreds of biologically active substances that have a neurochemical effect through neuroendocrine, immune, and metabolic pathways. The microbiota also synthesizes and releases products (neurotoxins, neurotransmitters, lipopolysaccharides, amyloids, etc.) that can negatively affect the neurochemistry of the CNS, stimulating the development of amyloidosis, synucleinopathies, and tauopathies, thereby promoting the development and/or progression of neurodegenerative diseases. Metabolites produced by the altered microflora are able to enter the bloodstream and possibly into the CNS, thereby disrupting its functioning. Infections can play a significant role and even act as a cofactor in the induction of neurodegenerative diseases. Disturbance of the functions of the GI can precede long before the neurodegenerative processes. Early diagnosis, detection, monitoring, and treatment of negative gastrointestinal symptoms, including normalization of the microbiota, can lead to a significant improvement in the quality of life of patients with neurodegenerative diseases.

This work was supported by a state contract with the Federal Agency for Scientific Organizations of Russia (project no. AAA-A18-118012290371-3)

ROLE OF CORTICOSTERONE IN THE HIPPOCAMPAL DAMAGE IN LATE PERIOD OF TRAUMATIC BRAIN INJURY IN RATS

Kostyunina O.V.[1], Volkova A.A.[1,2], Komoltsev I.G.[1,3], Shirobokova N.I.[1], Frankevich S.O.[1,3], Salyp O.Y.[1], Bashkatova D.A.[1], Shalneva D.V.[1], Chizhova O.A.[1], Kostrukov P.A.[1], Novikova M.R.[1], Gulyaeva N.V.[1,3]

1 - Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow, Russia
2 - Veltischev Research and Clinical Institute for Pediatrics of the Pirogov Russian National Research Medical University, Moscow, Russia
3 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

okostyunina67@mail.ru

Introduction. Traumatic brain injury (TBI) pathogenesis is known to be mediated by neuroinflammation and neurodegeneration. In the hippocampus, a selectively vulnerable area, glucocorticoid receptors are highly expressed and regulate stress response. Acute and chronic corticosterone (CS) elevations may be involved in early hippocampal damage after traumatic brain injury (TBI) and further secondary neurodegeneration, a putative morphological substrate for late TBI sequelae. In this study we aimed to evaluate CS-dependent morphological changes 3 months after TBI.

Materials and methods. The study was performed on 51 male Sprague-Dawley rats, divided into TBI, sham and control groups. TBI was modeled using lateral fluid percussion. CS levels in blood were assessed by ELISA before craniotomy, on days 3, 7, and 3 months after TBI. Anti-GFAP and anti-Iba1 staining was used for the histological analysis. The average number of microglial and astroglial cells was calculated in sections of the ipsilateral and contralateral hippocampal dentate gyrus (DG), CA1 and CA3.

Results. CS was elevated on day 3 after TBI and predicted remote mortality. Three months after TBI neuronal cell loss in the ipsilateral DG, bilateral thinning of hippocampal cell layers and astrogliosis were revealed. Unexpectedly, no differences in the number of microglial cells between hemispheres and groups were found. Acute CS elevation did not correlate with the hippocampal damage. Blood CS 3 months after TBI negatively correlated with microglial cell density in CA1 ipsilaterally ($p<0.017$) and CA3 area bilaterally ($p<0.02$).

Conclusion. TBI leads to hippocampal damage evident after 3 months. Early CS elevation is not directly associated with this damage. Blood CS levels during late posttraumatic period negatively correlate with microglial cell density, presumably mediating anti-inflammatory effects in survived rats.

Supported by RSF, grant № 22-25-00713.

ROLE OF THE NEUROPEPTIDASE NEPRILYSIN IN AMYLOID CLEARANCE

Nalivaeva N.N.[1,2,3]

1 - I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, RAS, St. Patarsburg, Russia
2 - I.P. Pavlov Institute of Physiology, RAS, St. Petersburg, Russia
3 - School of Biomedical Sciences, University of Leeds, Leeds, UK

natalia.nalivaeva@outlook.com

Accumulation of toxic species of amyloid Aβ peptide underlies pathogenesis of Alzheimer's disease (AD). While in the early onset familial forms of AD accelerated Aβ accumulation in the brain is caused by mutations in the amyloid precursor protein (APP) and presenilin genes, in the late onset sporadic form it is most likely caused by a deficit in the systems of amyloid clearance. Apart from various cellular (microglial and autophagic) and transport mechanisms (ApoE, transthyretin) of Aβ clearance, there is a wide spectrum of proteolytic enzymes which cleave Aβ in various brain regions. An endopeptidase neprilysin (NEP), degrading a variety of biologically active peptides, is also a major Aβ-degrading enzyme. Deficit of NEP expression and activity was shown to lead to accumulation of Aβ in the brain whereas up-regulation of its expression and enhancement of its activity increases amyloid clearance and improves cognitive deficits caused by Aβ toxicity. NEP gene expression decreases in cortical brain structures with ageing and, also, after hypoxic and ischemic insults which might predispose to AD development. Our studies have shown that the NEP gene is regulated by the C-terminal fragment of APP (AICD) produced alongside Aβ in the amyloidogenic pathway by β- and γ-secretase APP cleavages. Binding of AICD to the NEP gene promoter depends on chromatin folding regulated by histone deacetylases (HDACs). Inhibition of HDACs by trichostatin A or valproic acid (VA) results in increased occupancy of AICD on the NEP promoter and upregulation of its expression. Following this regulation mechanism, we have demonstrated that NEP upregulation can be achieved by a variety of pharmacological agents (VA, tyrosine kinases and caspase inhibitors, nuclear retinoid X receptor agonists and an antioxidant epigallocatechin gallate) both in cell and animal models which widens therapeutic avenues in prevention and treatment of AD.

Supported: RFFI-19-015-00232, Russian state budget (assignment 075-00408-21-00).

SEROTONIN 5-HT2A AND TRKB RECEPTORS FORM OLIGOMERIC HETEROCOMPLEXES IN VITRO AND IN VIVO

Ilchibaeva T.V.[1], Tsybko A.S.[1], Zeug A.[2], Ponimaskin E.[2], Naumenko V.S.[1]

1 - Institute of Cytology and Genetics, Siberian Branch of RAS, Novosibisk, Russia
2 - Medical School Hannover, Hannover, Germany

rbicehok@mail.ru

Serotonin 5-HT_{2A} receptor and brain-derived neurotrophic factor (BDNF) receptor TrkB are broadly expressed in different brain regions where they are critically involved in regulation of neuroplasticity in health and disease. Cross-talk between the BDNF and 5-HT_{2A} receptors was demonstrated in the number of studies. At the same time, an exact mechanism of modulation of BDNF system through 5-HT_{2A} receptors is still unknown. TrkB receptor may be one of the possible targets for realization of this interaction. So, the aim of the present study was to examine whether 5-HT_{2A} and TrkB receptors are able to form heteroreceptor complexes both *in vitro* and *in vivo*.

Using co-transfection of N1E-115 cells with 5-HT_{2A} and TrkB receptors followed by CO-IP the presence of heteroreceptor complexes was shown. We analyzed the interaction between 5-HT_{2A} and TrkB receptors in living cells with a lux-FRET approach. The lux-FRET analysis revealed high apparent FRET efficiency for 5-HT_{2A}R-mTurqouise2 and TrkB-YPet. Using CO-IP, we have identified 5-HT_{2A}-TrkB oligomeric complexes in the samples from the frontal cortex, hippocampus and striatum of mice. As it was shown by immunohistochemistry, 5-HT_{2A} and TrkB receptors were highly co-localized in all investigated brain regions. Nerve cells of dorsal hippocampus as well as cortical and striatal cells are shown to be PLA-positive. The ratio of PLA signal to number of cells was significantly higher in the hippocampal cells demonstrating prevalence of 5-HT_{2A}-TrkB heteromeric complexes in this brain region compared to those in the frontal cortex ($p<0.05$) and striatum ($p<0.01$).

Thus, we have shown for the first time that 5-HT_{2A} receptors can form heterodimers with TrkB receptors both *in vitro* and *in vivo*. Obtained results indicate a new molecular mechanism that could play an important role in the brain functioning.

The work supported by the Russian Foundation for Basic Research (#20-04-00253) and the Basic Research Project (#0259-2021-0015).

SIGMA-1 RECEPTOR – A ROLE IN NEURONAL SIGNALING AND NEURODEGENERATION

Bezprozvanny I.[1,2]

1 - Peter the Great St Petersburg Polytechnical University, St Petersburg, Russia
2 - Southern Federal University, Rostiv-on-Don, St. Peterburg, Russia

Mnlabspb@gmail.com

The sigma 1 receptor (S1R) is a 223 amino acid-long transmembrane endoplasmic reticulum (ER) protein. Agonists of S1R demonstrated neuroprotective effects in variety of preclinical models and there are several on-going clinical trials of S1R agonists in neurodegenerative disorders. However, signaling functions of S1R are poorly understood. In our recent studies we tested the hypothesis that biological activity of S1R in cells can be explained by its ability to interact with cholesterol. By performing experiments in reduced reconstitution systems, we demonstrate direct effects of cholesterol on S1R clustering. We identify a novel cholesterol-binding motif in the transmembrane region of human S1R. Mutations of this motif impair association of recombinant S1R with cholesterol beads, affect S1R clustering in vitro and disrupt S1R subcellular localization. Further, we found that S1R agonists cause disruption of S1R clusters. Based on these results we propose that S1R-cholesterol interactions enable the formation of cholesterol-enriched microdomains in the ER membrane. We hypothesize that a number of secreted and signaling proteins are recruited and retained in these microdomains. This hypothesis is consistent with the results of an unbiased screen for S1R-interacting partners which we performed using the APEX technology. We further propose that S1R agonists enable the disassembly of these cholesterol-enriched microdomains and the release of accumulated proteins such as ion channels, signaling receptors, and trophic factors from the ER. We also propose that these cholesterol-enriched microdomains form the basis for formation of membrane contact sites between ER and other subcellular organells such as mitochondria and plasma membrane.

Literature

1.Ryskamp at al (2017) The sigma-1 receptor mediates the beneficial effects of pridopidine in a mouse model of Huntington disease. Neurobiol Dis. 97: p. 46-59.

2.Ryskamp at al (2019) Pridopidine stabilizes mushroom spines in mouse models of Alzheimer's disease by acting on the sigma-1 receptor. Neurobiol Dis. 124: p. 489-504.

3 Zhemkov at al (2021) The role of sigma 1 receptor in organization of endoplasmic reticulum signaling microdomains. Elife. 10.

4. Zhemkov at al (2021) Sigma-1 Receptor (S1R) Interaction with Cholesterol: Mechanisms of S1R Activation and Its Role in Neurodegenerative Diseases. Int J Mol Sci. 22(8).

5. Zhemkov, V., J. Liou, and I. Bezprozvanny. (2021) Sigma 1 Receptor, Cholesterol and

Endoplasmic Reticulum Contact Sites. Contact. 4: p. 1-7.

Supported by: Russian Science Foundation Grant 19-15-00184 and by the National Institutes of Health grants R01NS056224 and R01AG055577 (IB).

SIMULTANEOUS MONITORING OF THE INTRACELLULAR CL- AND PH CHANGES IN NEURONS OF TRANSGENIC MICE

Ponomareva D.N.[1,2,3], Petukhova E.O.[1], Bregestovski P.D.[2,3]

1 - Institute of Neurosciences, Kazan State Medical University, Kazan, Russia
2 - Department of Normal Physiology, Kazan State Medical University, Kazan, Russia
3 - Institut de Neurosciences des Systèmes, Aix-Marseille University, INSERM, INS, Marseille, France

ponomareva_dn@mail.ru

This study is devoted to fluorescent analysis of intracellular chloride (Cl^-) and pH in brain slices of recently developed transgenic mice expressing biosensor of Cl^- and H^+ ions (ClopHensor). The effects of ions modulating the activity of Cl^- and H^+ transporters on the intracellular Cl^- and H^+ transients were studied. We analyzed contribution of Ca^{2+}, Na^+ and Cl^- ions, and the effect of $GABA_A$ receptors suppression.

High frequency synaptic stimulation and elevation of extracellular K^+ (up to 20 mM) caused an intracellular increase of both ions, Cl^- and H^+. Upon application of bicuculline the mean amplitude of synaptically induced Cl^- influx decreased to 41.2±12.2% (n=7, $p<0.01$), pH transients simultaneously increased by 90.7±17.4% (n=8, $p<0.01$). The mean τ_{decay} of Cl^- transients was 35±4.2 s (n=9), that in 4.5 times shorter than the pH (157±20.3 s, n=16).

Applying a Ca^{2+}-free ACSF did not change the base level of Cl^-, but decreased pH by 0.09±0.02 pH (n=8, $p<0.01$). After adding the "low-Cl^-" ACSF, a diminishing base level of Cl^- by 7.1±1.1 mM ($p<0.05$, n=5), synchronously with alkalization of neurons by 0.19±0.01 pH ($p<0.05$, n=5) was observed. The pH transients induced by high K^+ concentration or by synaptic stimulation in "low Cl^-" ACSF conditions increased by 2 and 5 times respectively, but Cl^- transients were nearly completely suppressed.

Changing the solution to a low-Na^+ ASCF reduced the intracellular Cl^- by 1.98±0.15 mM (n=5, $p<0.05$) and H^+ by 0.08±0.01 pH (n=5, $p<0.05$). In low-Na^+ ASCF, responses to K^+-induced depolarization were potentiated by more than 2 times for both ions.

Our observations demonstrate the efficiency of ChlopHensor transgenic mice for reliable non-invasive monitoring of intracellular Cl^- and pH in normal and pathological conditions.

Supported by the RSF Grant: 18-15-00313.

SCANNING ION CONDUCTANCE MICROSCOPY FOR INVESTIGATION OF CELLULAR MECHANISMS OF REGULATION IN THE NERVOUS SYSTEM

Gorelkin P.V.[1], Kolmogorov V.S.[1], Erofeev A.S.[1], Korchev Y.E.[1,2]

1 - National University of Science and Technology "MISiS", Moscow, Russia
2 - Imperial College London, London, UK

gorelkin.pv@misis.ru

In the last few decades, a micropipette has become an irreplaceable tool in cell physiology. It has been widely used for local application of chemicals, rapid perfusion application, intra- and extracellular perfusions and voltage measurements, whole cell and single channel current recording, etc. Many breakthrough discoveries leading to a better understanding of cell physiology and cell functions have been made using this tool. The functionality of this rather simple tool has been dramatically expanded with miniaturisation of the pipette tip down to nanometre scale, and invention of scanning ion conductance microscopy (SICM). SICM uses ion current flowing out of the tip of a glass nanopipette (pipette with tip diameter about 100 nm) filled with electrolyte to detect presence of a surface in a noncontact fashion. By scanning the sample in x-y plane and moving the nanopipette up and down on the z-axis the SICM recreates 3D topography image of the sample surface. We have shown that scanning nanopipettes are capable of reproducing 3D topography of complex, soft, live biological samples such as neuronal networks at resolution better than atomic force microscopy.

We have successfully combined scanning nanopipettes with principles of electrophysiology, fluorescence microscopy, electrochemistry, electrophoresis, and electroosmosis to investigate single channels and receptors in cellular membranes, to deliver biomolecules to precisely defined locations in cell cultures, and to perform single cell analysis.

Mechanical properties of living cells determined by cytoskeletal elements play a crucial role in a wide range of biological functions. SICM can been used for quantitative nanomechanical mapping based on intrinsic force interactions between nanopipettes and samples. We have demonstrated the application of SICM for nanomechanical mapping of living neuronal cells treated with oligomers of amyloid beta to investigate the influence of protein misfolding on cellular mechanisms of neurodegenerative disease progression.

Supported by the RSF Grant 20-14-00312.

SEARCHING FOR NONCANONICAL DNA REPAIR MECHANISMS IN NEURONS

Fortygina P.A., Beletsky A.P., Chesnokova E.A., Zolotar A.N., Kolosov P.M.

Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences, Moscow, Russia

peterkolosov@gmail.com

Neuronal activity may cause formation of DNA double-strand breaks (DSB), which contributes to the regulation of gene expression. The absence of homologous recombination in neurons suggests the existence of unusual repair mechanisms. In this work we explored the possibility of RNA-dependent DNA repair in neurons. Using immunocytochemistry, we estimated localization of some proteins, associated with DSB repair or RNA-dependent DNA synthesis, in cultured rat hippocampal neurons. We visualized γH2X, a known DSB marker, RAD52, a recombinase that binds a break site with a homologous template, and two LINE1 retrotransposon proteins: ORF1p (RNA-binding chaperone) and ORF2p (endonuclease/reverse transcriptase).

RAD52 protein and its phosphorylated form p-RAD52 - cells with neuronal and glial morphology both displayed RAD52 staining in the cytoplasm. Only cells with neuronal morphology showed RAD52 localization in prominent nuclear clusters. Localization of RAD52 clusters was usually mutually exclusive with the fluorescent signal of DAPI, meaning that RAD52 is predominantly located in interchromatin and hyper-acetylated euchromatin zones. To test the involvement of RAD52 in repair of artificially induced DSBs, etoposide (a topoisomerase II inhibitor) was added to the cultures. Increased number of RAD52 nuclear clusters and increased fluorescence intensity of RAD52 (30%, $p=0.042$) and p-RAD52 were observed under conditions of DNA damage, suggesting an important role of RAD52 in DNA repair in neurons. These results are consistent with the reported role of RAD52 in repair of transcriptionally active genes.

ORF1p was stained in the cytoplasm of the studied cells, while ORF2p was found in the nuclei and the cytoplasm. Localization patterns of these proteins in neurons are different from patterns reported for other cell types. This suggests that LINE1-encoded proteins may have specific functions in neurons, possibly related to RNA-based DNA repair.

In conclusion, nuclear co-localization of RAD52 and ORF2p observed in some cells raises the possibility of their cooperative activity in an RNA-dependent DNA-repair mechanism. These preliminary results require further investigation.

SEPARATION OF THE Ca^{2+} RESPONSE OF ASTROCYTES AND NEURONS IN RODENT CORTICAL CULTURE WITH GLUTAMATE EXCITOTOXICITY

Tarzhanov I.A.[1,2], Zgodova A.E.[1,2], Bakaeva Z.V.[1], Surin A.M.[1]

1 - Children's Health Research Center of the Ministry of Health of the Russian Federation, Moscow, Russia
2 - Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University), Moscow, Russia

likeman.1998@yandex.ru

Glutamate (Glu) excitoxicity, caused by hyperstimulation of Glu ionotropic receptors and subsequent Ca^{2+} entry into neurons, is a key event in the pathophysiology of stroke and other CNS diseases. When modeling brain processes *in vitro*, it is important to separate the contribution of glial cells and neurons. The aim of this work is to study the effect of the culture cellular composition on the $[Ca^{2+}]_i$ change under the action of toxic Glu concentrations.

Cell cultures were obtained from the newborn Wistar rats cerebral cortex and kept at 37°C, 100% humidity, and 5% CO_2 for 7-11 days *in vitro*. The glia cells proportion was regulated by inhibiting astrocyte proliferation using Cytarabine (ara-C) (2 μm), added 48-72 hours after planting. Fluorescence measurements were performed using a Nikon Ti-2 inverted fluorescence microscope and a complex of multi-wave excitation and signal recording. $[Ca^{2+}]_i$ changes were recorded using the Fura-FF indicator.

According to the literature [1], the Ca^{2+} release from the endoplasmic reticulum under the action of ATP on astrocytes purinergic receptors enables differentiate them in neuroglial culture. Besides, adding of KCl opens potential-dependent Ca^{2+} channels in neurons. We found that in cultures without the ara-C addition the proportion of cells, rising $[Ca^{2+}]_i$ in respond to ATP increased from 2-5% to 20%. The cultivation with ara-C increased the number of cells that respond with the $[Ca^{2+}]_i$ rise to KCl from 45 to 60%. The older the culture, the more sensitive the neurons were to Glu. The gradual Glu receptors expression in neurons were shown in the literature [2]. Without ara-C the proportion of neurons, developing Delayed Calcium Dysregulation under the Glu action, decreased, suggesting astrocytes protective effect. However, it is necessary to keep in mind the toxic effect of ara-C itself.

Thus, the ATP addition enabled to determine astrocytes presence in culture when the KCl action stimulates both cell types.

Literature:

1. P. R. Angelova et al., The Journal of Neuroscience, 2015
2. A. E. King et al. J. Comp. Neurol., 2006

SNIFFING LUNG CANCER VOLATILE FINGERPRINTS BY CALCIUM IMAGING IN THE GLOMERULAR LAYER OF THE RAT OLFACTORY BULB

Lysenko L.V., Romanyuk R.A., Matukhno A.E., Petrushan M.V.

Southern Federal University, Rostov-on-Don, Russia

lalv@sfedu.ru

Lung cancer (LC) remains the leading cause of cancer mortality, but its early-stage diagnosis still challenging, due to the lack of specific syndromes and a limited understanding of etiology. Detection of specific volatile organic compounds (VOC) in the exhaled breath of patients enables the introduction of adequate treatment without delay, which is key to improving survival. The success of VOC-based diagnosis depends on the identification of chemical species that can serve as biomarkers of LC. Here we examined the potential of calcium imaging in the rat olfactory bulb to detect LC volatile fingerprints in exhaled air samples. Ten healthy nonsmoking volunteers (44±6 y.o.) were involved in the study. Two samples of exhaled air were collected into 10 L Tedlar sampling bags from each volunteer: one control, the other, with LC biomarkers (cyclohexyl isothiocyanate, benzene, 50 ppb) added. Two-photon calcium imaging of odor-evoked glomerular activity was performed on 6-month-old male Rattus norvegicus rats. We employed a deep learning-based method using a convolution neural network (CNN) with triplet loss and metrical classification as core methods for decoding the patterns of glomerular maps to determine biomarkers in air samples. To obtain a sufficient training dataset, we generated datasets of artificial glomerular activity maps based on empirically observed qualities inherent to experimentally obtained olfactory activity maps. Triplet loss-based CNN classification method with pretraining on synthetic dataset turned out to be appropriate to detect biomarkers in human breath with accuracy up to 81 %. These results suggest that detection of specific biomarkers by analysis of patterns of calcium activity, evoked by human exhaled air in rat olfactory bulb, may potentially identify patients with LC and could be regarded as a new promising approach for early LC detection.

Supported by the Russian Science Foundation, grant # 22-25-00683 for the Southern Federal University, https://rscf.ru/project/22-25-00683/

STEVIA REBAUDIANA PROTECTS AGAINST ROS-INDUCED TOXICITY FOLLOWING SPINAL CORD INJURY

Simonyan K.V.[1], Avetisyan L.G.[1], Isoyan A.S.[1], Hovhannisyan L.E.[2], Simonyan R.M.[3], Chavushyan V.A.[1]

1 - Orbeli Institute of Physiology NAS RA, Yerevan, Armenia
2 - G.S.Davtyan Institute of Hydroponics Problems NAS RA, Yerevan, Armenia
3 - H. Buniatian Institute of Biochemistry NAS RA, Yerevan, Armenia

karensimonyan86@gmail.com

Spinal cord injury (SCI) is associated with the generation of reactive oxygen species (ROS) and oxidative damage caused by oxygen free radicals. Within the first minutes and hours after injury, a number of sources of the primordial free radical superoxide (O2 •-) may be active in the injured spinal cord. NADPH oxidase (NOX) is a multisubunit enzyme that catalyzes the reduction of molecular oxygen to produce superoxide using NADH or NADPH as the electron donor. NOX and ROS are age-related mediators of SCI pathophysiology, and normally protective M2 macrophages may potentiate secondary injury in the aged injured spinal cord via ROS generation. We isolated and purified for the first time the stable superoxide producing associate of NOX with NADPH containing lipoprotein of Stevia leaves cell membranes. According to preliminary findings, the NADPH-containing lipoprotein biomembrane of both animal and plant origin is a cofactor for NOX located on the same membranes. Traditional and herbal therapies are gradually gaining popularity and molecules found in plants have been shown to be antioxidant in nature. We used Armenian Stevia rebaudiana, which is high in diterpene glycosides, extractive substances, and beneficial macro-microelements (K, Ca, Mg, Zn, Fe, Cu). We discovered that Stevia rebaudiana reduced ROS production in the spinal cord and improved functional recovery after SCI. Stevia rebaudiana was found to prevent increased ROS production in the spinal cord and improve functional recovery following hemisection of spinal cord in rats.

This work was supported by the State Committee of Science RA (Research project 19YR-1F010).

STUDY OF OXIDATIVE STRESS OF SINGLE NEURONAL CELLS TREATED BY FLUORESCENTLY LABELED AMYLOID

Timoshenko R.V.[1], Vaneev A.N.[1,2], Savchenko A.G.[1], Majouga A.G.[1,2], Korchev Y.E.[1,3], Gorelkin P.V.[1], Erofeev A.S.[1,2]

1 - National University of Science and Technology «MISIS», Moscow, Russia
2 - Lomonosov Moscow State University, Moscow, Russia
3 - Imperial College London, London, UK

timoshenkoroma@mail.ru

Neurodegeneration is characterized by progressive loss of structure and/or function of neuronal cells. Mitochondrial dysfunction and oxidative stress have been shown to contribute to the pathogenesis of neurodegenerative disorders, including Alzheimer's disease (AD). AD is the most common cause of dementia in the elderly and is characterized by the accumulation of neurofibrillary tangles and amyloid plaques in the brain, resulting in neuronal and synaptic dysfunction. Buildup of amyloid peptides (Aβ), a major component of amyloid plaques, can adversely affect mitochondria and promote metabolic dysfunction, thereby implicating mitochondria in AD pathogenesis. A big step towards understanding AD will be the study of oxidation stress processes of living single cells. Today, this is quite difficult to do due to the lack of simple and low-invasive measurement methods. In this work, we studied the effect of fluorescently labeled amyloid Aβ42 on single neuronal cells using low-invasive Pt-nanoelectrodes.

First, we prepared a series of quartz nanocapillaries, which were modified with pyrolytic carbon and platinum to increase the catalytic activity of the sensor. It was used to measure intracellular ROS level in single SH-SY5Y (neuroblastoma) cells treated by FAM-labelled Aβ42. We noticed that cells with formed amyloid aggregates had higher level of ROS (35 ± 4 μM) compared with cells without formed aggregates, which are shown oxidative stress too (13 ± 3 μM). Untreated cells were used as a control (7 ± 1 μM). Thus, for the first time, intracellular levels of ROS were electrochemically measured in single neuronal cells treated with beta-amyloid.

This work was supported by the Russian Science Foundation, № 19-79-30062

STUDY OF THE EFFECT OF CONSUMED ETHANOL SOLUTION ON THE LEVEL OF TYROSINE HYDROXYLASE IN THE NUCLEUS ACCUMBENS IN MALE DAT-HET RATS UNDER FREE ALCOHOLIZATION

Antonova I.V., Veraksa A.E.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

risha.irina999@mail.ru

The dopamine system directly affects the formation of alcohol abuse, as it is part of the system of reward. Dysregulation of the mesolimbic part of the dopamine system is a risk factor for increased alcohol consumption (Engel J.A., Jerlhag E. 2014). The role of dopamine in the formation of alcohol preference may be associated with general changes in catecholamine metabolism. Tyrosine hydroxylase (TH) is the main enzyme in catecholamine biosynthesis.

We used male heterozygous knockout rats for the gene encoding the DAT transporter with increased levels of extracellular dopamine (DAT-HET, n=15), and male Wistar rats (n=8).

For study the localization of dopaminergic neurons in the nucleus accumbens area (nAcc), the immunohistochemical (IHC) method of TH localization was used.

We performed free alcoholization for 4 months. The experimental DAT-HET and Wistar groups got water and 10% ethanol solution as a drinking solution, the control group of DAT-HET rats got only water.

Analysis of the results of the alcohol solution drunk at the end of the experiment showed a wide variation in its level of consumption. Therefore, the animals were divided according to the amount of alcohol solution drunk. The sign (+) are rats drinking solution higher than the group average, the sign (-) are rats with low level of alcohol solution intake.

Differences were found in the amount of TH in the nAcc area: DAT-HET(+) ($p=0.004$) and DAT-HET(-) rats had lower tyrosine hydroxylase levels than the DAT-HET control group ($p=0.0003$). DAT-HET(-) rats had lower TH levels compared to Wistar(-) rats ($p=0.004$). The findings may suggest that the mechanism of the dopamine reward system in DAT-HET rats is not the main mechanism in the formation of alcohol preference.

STUDY OF THE SELECTIVE BLOCKER OF SODIUM-CALCIUM EXCHANGER KB-R7943 AS AN ANALGESIC DRUG IN RATS WITH INDUCED TYPE 1 DIABETES MELLITUS

Sukhov I.B.[1], Dashieva V.Zh.[2,1], Chistyakova O.V.[1], Spasova A.P.[3], Ivanova N.[4,5], Gateva P.A.[4], Shestakova N.N.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St. Petersburg, Russia
2 - Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
3 - Petrozavodsk State University, Petrozavodsk, Russia
4 - Medical University Sofia, Faculty of Medicine, Sofia, Bulgaria
5 - Institute of Neurobiology, Bulgarian Academy of Sciences (BAS), Sofia, Bulgaria

sukhov.ivan@gmail.com

NMDA receptors (NMDAR) play a primary role in neuropathic pain pathogenesis. However, direct blocking of NMDAR leading to pain relief has a number of negative side effects and is poorly tolerated by patients. The sodium-calcium exchanger (NCX) is involved in the process of calcium-dependent desensitization of NMDAR and is considered by us as a target for indirect action on NMDAR to achieve an analgesic effect in diabetic neuropathy.

The aim is to study the possibility of using selective NCX blockers for the development of effective and safe analgesic therapy using KB-R7943 as example.

Methods. Behavioral tests Randall-Selitto and Cold plate, ECG. The experiments were carried out on 4-month male Wistar rats; type 1 diabetes mellitus (DM1) was induced by STZ; study drugs were administered perorally at 10 mg/kg dose for 7 days; for comparison, amitriptyline (ATL), the first-line antidepressant for neuropathic pain relief, was used.

Results. The Randall-Selitto and Cold plate tests found an increase in pain threshold in DM1 animals and a comparable analgesic effect after KB-R7943 and ATL therapies.

ECG registration was performed in 55 animals divided into *the following* 6 groups: DM1 (n=28), two of which were administered ATL (n=9), KB-R7943 (n=9); and 3 groups of healthy rats (n=27), two of which received study drugs (n=7 and n=9, respectively).

Analysis of ECG parameters confirmed complications in *the* DM1 group: an increase in the duration of intervals QT by 18%, RR by 15%, RT by 37%, and RTc by 31% with a decrease in the average heart rate by 11%.

After administration of ATL to healthy rats, a significant increase in RTc interval by 28% was found. In DM1 group, the negative ATL impact worsened: S-wave increased by 2.1 times, RT interval by 24%, the area under the T-wave to the peak by 2.4 times, the area under T-tooth by 2.06 times.

KB-R7943 administration to both healthy and DM1 animals had no effect on ECG parameters and heart rate variability, which indicates the absence of side effects on the

cardiovascular system in it using.

Conclusion. The development of pain therapy based on selective NCX blockers is promising.

RFBR grant 20-515-18008, NSFB Grant КП-06-Rusia 25/16.12.2020.

STUDYING AMYLOID AGGREGATES FORMATION ON LIVING CELL SURFACE BY SCANNING ION-CONDUCTANCE MICROSCOPY

Kolmogorov V.S.[1,2], Erofeev A.S.[1], Timoshenko R.V.[1], Lopatukhina E.V.[1], Barykin E.P.[3], Kozin S.A.[3], Klyachko N.L.[2], Mitkevich V.A.[3], Majouga A.G.[4], Makarov A.A.[3], Edwards C.[5], Korchev Y.E.[1,5], Gorelkin P.V.[1]

1 - National University of Science and Technology "MISIS", Moscow, Russia
2 - Lomonosov Moscow State University, Moscow, Russia
3 - Engelhardt Institute of Molecular Biology of Russian Academy of Sciences, Moscow, Russia
4 - D. Mendeleev University of Chemical Technology of Russia, Moscow, Russia
5 - Imperial College London, Department of Medicine, London, UK

vskolmogorov@gmail.com

Formation of toxic protein aggregates that cause dysfunction and death of neuron cells is a main reason of neurodegenerative diseases such as Alzheimer's disease (AD). Neurodegeneration is preceded by synaptic disorders connected with alterations in the membrane ionic conductivity of neuronal cells. It has been demonstrated that receptor-mediated toxicity and disruption of ion channel induce subsequent stages of the pathogenic cascade, such as pathology of tau proteins and neuroinflammation in AD. Also, influence of amyloid aggregates on the cytoskeleton and consequently on the mechanical properties due to the activation of the kinases LIMK1, p38MAPK, CAMKII, Rho/Cdc42 GTPases is widely supported. It's possible to characterize such alterations of local mechanical properties qualitatively and quantitatively by using scanning ion-conductance microscopy (SICM).

Recently we have demonstrated [Kolmogorov et al., Nanoscale, 2021] the possibility of correlative topography and Young's modulus mapping with simulations of confocal visualization of living cells by SICM. In this work novel technique was used to observe formation of fluorescently labelled amyloid aggregates on living SH-SY5Y cells. We showed that aggregation of β-amyloid preceded by dramatic increase of Young's modulus on whole cell and local decrease of it on aggregation area specifically. Confocal visualization confirmed pore-like aggregation and local disruption of lipid membrane at this specific area. Moreover, this technique allowed to perform Patch-Clamp recording on living cell with evidently formed β-amyloid aggregate and observe reduced membrane potential. Thus, SICM may be successfully used for multimodal studying

cytotoxicity mechanisms of β-amyloid aggregates on living cells.

This research was funded by The Russian Science Foundation, grant number № 19-79-30062.

SUSCEPTIBILITY OF SOMATOSENSORY CORTEX TO CORTICAL SPREADING DEPRESSION AND EXCITABILITY OF TRIGEMINAL AFFERENTS OF RATS WITH PRENATAL HYPERHOMOCYSTEINEMIA

Sitdikova G.F.[1], Gerasimova E.V.[1,2], Koroleva K.S.[1], Ermakova E.V.[1]

1 - Kazan Federal University, Kazan, Russia
2 - Neuroscience Program, Sirius University, Sochi, Russia

sitdikovaguzel@gmail.com

Homocysteine is a sulfur-containing amino acid produced in the metabolic cycle of methionine, and demonstrated neurotoxic effects in high concentrations. Elevated level of homocysteine level in prenatal period induces developmental disorders and different neurological pathologies in postnatal life. Population data indicate that a high homocysteine level (hyperhomocysteinemia, hHCY) in plasma or cerebrospinal fluid is associated with a risk factor for migraine headaches. However, there is no experimental evidence for the involvement of this amino acid in the pathogenesis of migraine. Cortical spreading depression (CSD) is considered to be electrophysiological correlate of migraine with aura which frequency is associated with hHCY. The aim of our study was to analyze in the development of CSD in the somatosensory cortex of rats in vivo and the excitability of peripheral afferents of the trigeminal nerve and trigeminal ganglion neurons in vitro in rats with prenatal hHCY. We also analyzed anxiety levels, photophobia, and mechanical sensitivity in rats with prenatal hHCY. Our results demonstrated higher susceptibility of somatosensory cortex of rats with hHCY to generation of CSD evoked by KCl and higher rate of CSD propagation. The background neuronal activity and burst of MUA at the onset of CSD was higher in rats with hHCY. Rat with hHCY showed mechanical allodynia, photophobia and anxiety which also typical for patients suffering from migraine attacks. Analysis of the electrical activity of the trigeminal nerve and the intrinsic properties of trigeminal ganglion neurons revealed higher excitability of the peripheral afferents innervating the meninges, which nociceptive firing underlies headache pain. In conclusion our data provide experimental evidence of causal link between elevated level of homocysteine in plasms and high risk of migraine attacks.

This work was supported by the Russian Science Foundation (grant number 20-15-00100)

SYNAPTIC VESICLE ENDOCYTOSIS IN MOTOR NERVE ENDINGS OF FUS TRANSGENIC MICE WITH MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

Grigoryev P.N., Zefirov A.L., Mukhamedyarov M.A.

Kazan State Medical University, Kazan, Russia

grigorievp@yahoo.com

The transmitter release and synaptic vesicle endocytosis were investigated by microelectrode intracellular recording of postsynaptic potentials and fluorescent confocal microscopy at mouse diaphragm motor nerve endings of transgenic mice with a model of amyotrophic lateral sclerosis. Transgenic FUS1-513 mice with ectopic neurospecific expression of the shortened human FUS gene on a CD1 genetic background (FUS-mouse) at the age of 40-60 days (an early presymptomatic stage of the disease) were used. Wild-type mice (WT mice) of the same age were used as controls. End-plate potentials (EPP) and miniature EPP were recorded by glass intracellular microelectrodes. The fluorescent dye FM 1-43 (6 mkM) to investigate synaptic vesicle endocytosis was used. Quantal content of EPP under stimulation of the motor nerve in transgenic FUS mice was lower than in WT mice. High-frequency stimulation (50 Hz) of the motor nerve in the presence of FM 1-43 lead to the dye loading into nerve endings by synaptic vesicle endocytosis. The fluorescent dye loading in transgenic FUS mice was reduced than in WT mice as the same stimulation time was used. But FM 1-43 loading was not significantly different in the case of usage of the different stimulation times in transgenic FUS and WT mice at which the approximately equal number of neurotransmitter quanta were released. Obtained data may indicate that synaptic vesicle exo-endocytosis intensity was decreased but the average synaptic vesicle recycling time was not greatly changed in FUS transgenic mice at an early presymptomatic stage of the disease.

The study was supported by RSF project 19-15-00329 and RFBR project № 20-015-00507.

THE ALLOSTERIC AGONISTS AND ANTAGONISTS OF THYROID-STIMULATING HORMONE RECEPTOR AND THEIR EFFECT ON THYROLIBERIN-STIMULATED THYROID HORMONES PRODUCTION

Fokina E.A.[1], Derkach K.V.[1], Bakhtyukov A.A.[1], Sorokoumov V.N.[1,2], Zakharova I.O.[1], Stepochkina A.M.[1], Shpakov A.O.[1]

1 - I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia
2 - Saint-Petersburg State University, Institute of Chemistry, St. Petersburg, Russia

fokina-katrina@yandex.ru

The thyroid-stimulating hormone (TSH) is the key regulator of the hypothalamic-pituitary-thyroid axis. In thyroid, TSH binds to G_s/G_q protein-coupled TSH receptor (TSHR) and stimulates the production of thyroid hormones. The development of specific agonists and antagonists of TSHR is an important approach to treat thyroid diseases, and the greatest interest among them are thieno[2,3-d]-pyrimidine-based allosteric TSHR regulators. The purpose of the study was to synthesize and study the biological activity of thieno[2,3-d]-pyrimidine derivatives with the activity of full and inverse agonists and neutral antagonists of TSHR. We synthesized three new compounds, such as 5-amino-*N*-(*tert*-butyl)-4-(4-iodophenyl)-2-(methylthio)thieno[2,3-d]-pyrimidine-6-carboxamide (1), 5-amino-*N*-(*tert*-butyl)-4-(4-(3-methoxyprop-1-yne-1-yl)phenyl)-2-(methylthio)thieno[2,3-d]-pyrimidine-6-carboxamide (2) and ethyl-2-(4-(4-(5-amino-6-(*tert*-butylcarbamoyl)-2-(methylthio)thieno[2,3-d]-pyrimidine-4-yl) phenyl)-*1H*-1,2,3-triazol-1-yl)acetate (3). Compounds 1 and 2 inhibited the TSH-stimulated synthesis of thyroid hormones in cultured FRTL-5 cells. Their administration to rats (15 mg/kg, i.p.) reduced thyroliberin-stimulated levels of thyroid hormones. In thyroid, compounds 1 and 2 inhibited the thyroliberin-stimulated expression of the *Tg*, *TPO* and *Dio2* genes encoding thyroglobulin, thyroperoxidase and D2-deiodinase. Compound 1 reduced the unstimulated level of thyroid hormones in FRTL-5 cells. In rats, compound 3 (15 mg/kg, i.p.) increased the plasma thyroid hormones level and thyroid expression of *Tg*, *TPO* and *Dio2* genes. Compound 3 increased the thyroliberin-stimulated production of thyroid hormones and the *TPO* and *Dio2* expression. Thus, we have developed new TSHR regulators with the activity of full agonist/positive modulator (3), inverse agonist (1), and neutral antagonist (2), which can be used to treat thyroid diseases.

This work was supported by the Russian Science Foundation (No. 19-75-20122).

THE EFFECT OF INHIBITION OF WATER CHANNEL AQP4 ON NIGROSTRIATAL NEURODEGENERATION UNDER BASAL CONDITIONS AND IN RAT MODEL OF PARKINSON'S DISEASE

Lapshina K.V., Abramova Yu.Yu., Guzeev M.A., Ekimova I.V.

I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

ksenia.lapshina@gmail.com

The glymphatic system provides the elimination of metabolites (particularly amyloid proteins) from the brain and its dysfunction can contribute to the progression of neurodegenerative diseases, including Parkinson's disease (PD). Water channel aquaporin 4 (AQP4) is a very important component of this mechanism. Our study was aimed to evaluate the effect of AQP4 inhibition on the development of neurodegeneration in the nigrostriatal system and motor functions under basal conditions and in the model of the preclinical stage of PD.

The experiments were carried out on male Wistar rats. AQP4 was inhibited using the inhibitor TGN-020 injected into the lateral ventricle of the brain. The model of the preclinical stage of PD was reproduced using the proteasome inhibitor lactacystin (LC) injected bilaterally into the SNpc. To assess the neurodegenerative changes and motor impairment the immuhistochemical methods and behavioral tests were applied.

We found that TGN-020 alone can induce dose-dependent neuronal death in SNpc. For use in further experiments we chose the dose which did not evoke significant neurodegeneration. The LC-induced model of PD was characterized by a loss of 20-30% of neurons in the SNpc in absence of motor disturbances. The pretreatment with TGN-020 increased the loss of SNpc neurons and their axons in the dorsal striatum by 1.6 times, led to a decrease in the level of tyrosine hydroxylase in SNpc neurons and caused motor dysfunction. Thus, the inhibition of AQP4 is able to evoke the more pronounced neurodegeneration and the appearance of motor dysfunctions in the model of PD. These patterns may reflect the transition of the preclinical stage of PD to the early clinical, and we suppose that Aqp4 can play an important role in the molecular mechanisms protecting brain functions from neurotoxic factors.

The study was supported by the RSF grant № 22-25-00607

THE MECHANISMS OF HIPPOCAMPAL SUSCEPTIBILITY TO HYPOXIA

Rybnikova E., Vetrovoy O.

Pavlov Institute of Physiology RAS, St. Petersburg, Russia

rybnikovaea@infran.ru

Hippocampus is known to be one of the most susceptible to hypoxic/ischemic damage brain regions. The mechanisms underlying selective vulnerability include glutamate excitotoxicity, hypermetabolism, calcium overload, mitochondrial zinc accumulation, glucocorticoid toxicity and neuroinflammation. Herein, we briefly summarize own contribution to dissecting of molecular basis of hippocampal vulnerability to hypoxia. We have been studied injurious effects of severe hypobaric hypoxia on brain neurons and demonstrated that greatest neuronal injury following hypoxia was observed in the hippocampal CA1 field. In an attempt to uncover possible mechanisms, we studied comparatively the patterns of expression and activity of the important regulatory molecules. The selective vulnerability of hippocampus, in particular CA1 field, in hypoxic conditions was accompanied by greater JNK1/2 activation, delayed up-regulation of *c-jun* mRNA and posphorylation of c-Jun, modified pattern of *ngfi-a* expression, down-regulation of Bcl-xL, changes in gluco-/mineralocorticoid receptors ratio. The pyramid neurons of CA1 had lowest basal levels of neurotrophin BDNF, more than twice lower than the granular cells of dentate gyrus. Studying hypoxia-inducible factor HIF-1 as a master regulator of pro-adaptive cellular response to hypoxic challenge, we found that the hippocampus differed from the neocortex by delayed (at 72 hours) or persistent (up to 72 hours) activation of hypoxia-inducible factor *hif-1α* gene and protein expression in response to severe hypoxia. Recently we have demonstrated that pharmacological inhibition of HIF-1 by a topotecan hydrochloride prevents neuronal apoptosis caused by severe hypoxia in hippocampus. These findings contradict the established theory about the pro-adaptive role of HIF-1 after hypoxia and confirm the hypothesis of its possible dual roles. HIF-1 inhibits expression and activity of pentose phosphate pathway enzymes necessary to maintain the antioxidant defenses, and down-regulates NRF2. This leads to decrease in NADPH and glutathione levels, promoting oxidative stress and triggering delayed neuronal loss. Supported by the Russian Science Foundation (grant № 22-25-00781).

THE NRF-2 ACTIVATOR PROTECTS CELLS WITH A MUTATION IN THE PINK1 GENE FROM THE ACUTE TOXIC EFFECTS OF HYDROGEN PEROXIDE

Kritskaya K.A., Fedotova E.I., Berezhnov A.V.

Institute of Cell Biophysics, Russian Academy of Sciences, Puschino, Russia

kritskayak96@yandex.ru

Parkinson's disease (PD) is one of the most common neurodegenerative diseases, accompanied by the death of neurons of the substantia nigra and the development of oxidative stress. Strategies for the treatment of oxidative stress with direct-acting antioxidants have not shown a significant effect in clinical studies of PD, and therefore it becomes urgent to search for the new protective effects that affect the antioxidant system of the cell. One of such approaches is an activation of Nrf-2 signaling pathway involved in transcription of genes of antioxidant response system of the cell.

To assess the viability of fibroblasts with a mutation in the gene encoding PINK1 (Homozygous p. Try90Leufsx12) obtained from a donor with established PD, the method of double staining (Hoechst 33342 – Propidium Iodide) was used. It was shown that under normal conditions, the viability of fibroblasts with PINK1 mutation does not differ from control cells, but under stress conditions (with the 24-h application of H_2O_2 250 uM), a significant decrease in the viability of fibroblasts with PINK1 mutation was found by 51.3% compared to control cells. Preliminary 24-hour incubation of cells with 50 nM of Nrf-2 activator RTA-408 increased the viability of fibroblasts with PINK1 mutation under stress conditions with hydrogen peroxide to the control level in cells without mutation.

The rate of ROS production was measured with H2DCF fluorescent probe. It was found that under normal conditions, the rate of ROS production in fibroblasts with PINK1 mutation is significantly (1.4 times) higher than in control. 24-hour preincubation of cells with 50 nM RTA-408 reduced the rate of ROS production in PINK1 cells to the level of control cells without mutation. Thus, activation of Nrf2 reduces the ROS-production and protects cells with PINK1 mutation under acute stress induced with H_2O_2.

The work was supported by the FEBS Summer Fellowship.

THE ROLE OF THE AUTOIMMUNE INFLAMMATION IN NEURONAL TISSUE IN DRUG-RESISTANT EPILEPSY

Bazhanova E.D.[1,2], Litovchenko A.V.[2], Zabrodskaya Yu.M.[3], Sitovskaya D.A.[3], Dryagina N.V.[3]

1 - Golikov Research Center of Toxicology, St. Petersburg, Russia
2 - Sechenov Institute of Evolutionary Physiology and Biochemistry Russia Academy of Sciences, St. Petersburg, Russia
3 - Almazov National Medical Research Centre, Polenov Neurosurgical Institute, St. Petersburg, Russia

bazhanovae@mail.ru

Temporal lobe epilepsy is considered to be the heaviest form of epilepsy, which cannot be treated by anticonvulsant drugs. It is known that inflammation play an important role in the epilepsy progression. Resistance to anticonvulsant drugs may be caused by neuroinflammation and autoimmunity, that damage AMPA and NMDA receptors.

Our aim was to identify autoimmune markers in blood and brain temporal cortex in patients with drug-resistant epilepsy.

We studied blood samples and biopsies of the brain temporal lobe in 80 patients with drug-resistant epilepsy. In the blood, anti-GAD, anti-GABA-R, anti-GRIN2A and anti-GLU concentrations were determined (ELISA). VEGF, CD45, CD3, CD8 expressions were determined in the brain biopsies (immunohistochemistry).

In the group of patients with drug-resistant epilepsy pronounced expression of VEGF was observed in the cytoplasm of endotheliocyte ($p<0.001$). The densitometric density of cells expressing VEGF was 0.41 ± 0.01, versus 0.19 ± 0.02 in control group. The total leukocyte antigen CD45 showed the presence of an inflammatory process in brain tissues present in all studied cases. CD3 (T-lymphocytes) expression was determined in 65% cases, CD8 (T-killers) - in 20%.

A slight decrease in the titer of autoantibodies to glutamate was recorded in our patients. A significant increase in the titer anti-NMDA was revealed compared with the control group. No difference was found in the titer of autoantibodies to GAD and GABA receptors.

Expressed VEGF promotes the mobilization of inflammatory cells to the site of injury. T-lymphocytes, including T-killers, play the main role in lymphocytic infiltration. Autoimmune T-lymphocytes spontaneously form in the immune system can penetrate through the damaged blood-brain barrier and deposited intensively in the brain. T-lymphocytes, or other immunocompetent cells provoke the expression of autoantibodies, which can have a direct proepileptogenic effect, or participate in the drug resistance development.

This research was funded by Russian Foundation for Basic Research (RFBR), grant № 20-015-00127.

TACHYPHYLAXIS OF THE ACID-SENSING ION CHANNEL ASIC1A

Komarova M.S., Bukharev A.R., Potapieva N.N., Tikhonov D.B.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

marrgosha4@gmail.com

Tachyphylaxis is a form of desensitization when the progressive decrease in response is seen after repetitive administration of a pharmacologically or physiologically active substance. This phenomenon is known for different types of ion channels but is poorly understood. Here we studied the tachyphylaxis in ASIC1a, the member of the proton-activated channels of the ASIC family. We made whole cell patch-clamp recordings from CHO cells, expressing rat ASIC1a homomeric channels. Previously tachyphylaxis of ASIC1a channels was considered slow desensitization, but here we demonstrated for the first time a partial reversibility of this effect. We have shown that slow (tachyphylaxis), acute and steady-state desensitization are independent processes and that slow desensitization occurs only when ASIC1a is in the open state. Also, we analyzed different factors affecting ASIC1a, such as potentiating or inhibiting drugs and pH conditions and have revealed a correlation between effects on ASIC1a response amplitude and the development of slow desensitization. Thus, tachyphylaxis is an important specific feature of homomeric ASIC1a channels and its characteristics, modulation, mechanisms and physiological significance deserve attention in further studies.

The work was supported by Russian Science Foundation grant 21-14-00280.

TAURINE PROTECTS AGAINST ALCOHOL-INDUCED STRUCTURAL CHANGES IN THE HIPPOCAMPUS IN RATS

Danielyan M.H., Karapetyan K.V., Nebogova K.A., Khachatryan V.P.

Orbeli Institute of Physiology NAS RA, Yerevan, Armenia

margaritadanielyan@rambler.ru

Hippocampal neurons have selective sensitivity to acute and chronic alcohol intoxication. This study aims at investigating the effect of the biologically active substance Taurine on the cellular structures of the hippocampus after alcohol intoxication. Chronic alcohol consumption resulted in reductions and changes in neuron number, volume, and size in the hippocampus. Degenerative changes in pyramidal cells with poorly visible processes, swollen granule cells of the dentate gyrus, and a decrease in hippocampal neuronal density were observed. Positive changes in

neuronal structure and an increase in phosphatase activity (increased metabolism) were observed in Taurine-treated rats' hippocampus. The findings suggest that Taurine has a neuroprotective effect on cellular structures in the rat hippocampus.

THE MECHANISM OF BRAIN-DERIVED NEUROTROPHIC FACTOR PRODOMAIN-INDUCED NEGATIVE REGULATION OF QUANTAL ACETYLCHOLINE RELEASE IN MAMMALIAN MATURE NEUROMUSCULAR SYNAPSES

Gaydukov A.E., Molchanova A.I., Alexashina S.V., Balezina O.P.

Lomonosov Moscow State University, Moscow, Russia

gaydukov@gmail.com

The effects of brain-derived neurotrophic factor (BDNF) processing by-product BDNF prodomain on the activity of mature neuromuscular junctions (NMJs) were studied in synapses of the mouse diaphragm.

The parameters of spontaneous miniature endplate potentials (MEPPs) and evoked endplate potentials (EPPs) were analyzed in presence of BDNF prodomain (1 nM). BDNF prodomain suppressed both spontaneous and evoked acetylcholine (Ach) release: decreased the frequency and amplitude of MEPPs, and the amplitude and quantal content of EPPs.

Inhibition of Rho-GDI-associated p75 receptor signaling with TAT-Pep5 peptide or sortilin with its inhibitor AF38469 completely abolished the BDNF prodomain-induced decrease in spontaneous and evoked quantal Ach release. Moreover, the downregulation of Ach induced by BDNF prodomain was prevented by by Rho-associated protein kinase (ROCK) inhibitor Y-27632. Taken together, these data suggest that in mature motor synapses activation of p75-sortilin by BDNF prodomain triggers the Rho-signaling pathway.

Tertiapin-Q, a G-protein-coupled inwardly rectifying potassium channels (GIRK) blocker, but not iberiotoxin, a BK-channels blocker, completely prevented the inhibitory effect of BDNF prodomain. SK-channels blocker apamin prevented the inhibitory effect of BDNF prodomain only partially. At the same time, the BDNF prodomain did not show any inhibitory effects in diaphragm motor synapses of pannexin 1 knockout mice, which have impaired purinergic regulation of neuromuscular transmission.

The data obtained suggest that there is a previously unknown mechanism for the acute suppression of spontaneous and evoked ACh release in mature motor synapses, which involves the activation of p75 receptors, ROCK and GIRK channels by BDNF prodomain and requires interaction with metabotropic purinoreceptors. In general, our results show that the product of

BDNF maturation has inhibitory effects on spontaneous and evoked ACh release in functionally mature neuromuscular junctions, which are mainly opposite to the effects of BDNF.

This work was supported by the Russian Science Foundation (Grant N 22-25-00111).

THE APPLICATION OF THE SELF-PROBING PRIMER PCR FOR QUANTITATIVE EXPRESSION ANALYSIS OF R607Q (UN)EDITED GLUA2 AMPA RECEPTOR MRNA

Schwarz A.P., Kovalenko A.A., Zakharova M.V., Zaitsev A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

aleksandr.pavlovich.schwarz@gmail.com

Adenosine deaminase-dependent RNA editing is a widespread universal mechanism of posttranscriptional gene function modulation. Changes in RNA editing level may contribute to various physiological and pathological processes. In the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor GluA2 subunit, A-I editing in the Q607R site leads to dramatic changes in function, making the receptor channel calcium-impermeable. A standard approach for quantifying (un)edited RNAs is based on endpoint PCR (Sanger sequencing or restriction analysis), a time-consuming and semiquantitative method. We aimed to develop RTqPCR assays to quantify rat Q607R (A-I) edited/unedited mRNA in samples in the present work. Based on self-probingPCR detection chemistry, described initially for detecting short DNA fragments, we designed and optimised RT-qPCR assays to quantify Q607R (un)edited mRNA. We used self-probing primer PCR technology for mRNA quantification for the first time. Using a novel assay, we confirmed that Q607R GluA2 mRNA editing was increased in 14-day- (P14) or 21-day-old (P21) postnatal brain tissue (hippocampus) compared to the embryonic brain (whole brains at E20) in Wistar rats. Q607R unedited GluA2 mRNA was detectable by our assay in the cDNA of mature brain tissue compared to that derived through classical methods. Thus, self-probing primer PCR detection chemistry is an easy-to-use approach for RT-qPCR analysis of RNA editing.

Supported by Russian Science Foundation, grant № 20-75-00127.

THE BLOCKADE OF SK AND KATP CHANNELS INCREASES THE NEURONAL FIRING FREQUENCY DURING THE SEIZURE-LIKE EVENTS BUT DOES NOT AFFECT THE EVOLUTION OF EPILEPTIFORM ACTIVITY IN THE ENTORHINAL CORTEX SLICE

Soboleva E.B., Amakhin D.V., Zaitsev A.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

soboleva.elena.1707@gmail.com

Small conductance $Ca^{(2+)}$-activated $K^{(+)}$ channels (SK) and ATP-dependent $K^{(+)}$ channels (KATP) are expressed in the entorhinal cortex neurons and can be upregulated during epileptic discharges, providing negative feedback and possibly serving as an intrinsic mechanism of seizure termination. Using the whole-cell patch-clamp recordings in rat brain slices, we investigated the role of these channels in the generation of seizure-like events (SLE) in the 4-aminopyridine model in vitro.

Bath application of apamin (SK-channel antagonist) or glibenclamide (KATP channel antagonist) did not change the frequency of SLEs during the 30 min of observation. Glibencalmide, but not apamin, induced a 25% increase of the SLE duration, indicating that KATP contribute to the termination of epileptiform discharges. Both these drugs altered the firing pattern of the principal neurons during the SLEs. The applied pharmaceuticals increased the depolarization during the SLE, which lead to a more pronounced depolarization block of action potential generation. During the late bursting phase of SLE, the average number of action potentials per burst was also increased in the presence of these drugs.

The obtained results indicate that the ion channels under consideration shape the neuronal firing during the epileptiform events but do not affect the general evolution of epileptiform activity in the entorhinal cortex slice at the large time scales. It can also be concluded that the enhanced depolarization block of the principal neurons does not affect the generation of the SLEs in the 4-aminopyridine model of epileptic seizures.

The research was funded by the Russian Science Foundation (project 21-15-00416).

THE CHANGES IN PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GENE EXPRESSION IN THE BRAIN OF RATS IN THE LITHIUM-PILOCARPINE MODEL OF EPILEPSY

Kovalenko A.A.[1], Zakharova M.V.[1], Schwarz A.P.[1], Dyomina A.V.[1], Roginskaya A.I.[1], Melik-Kasumov T.B.[2], Zubareva O.E.[1]

1 - Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia
2 - Institute of Physiology National Academy of Sciences of Belarus, Minsk, Belarus

ZubarevaOE@mail.ru

Currently, the role of gut-brain interactions in the pathogenesis of epilepsy is being actively discussed. Peroxisome proliferator-activated receptors (PPARs) play an important role in these interactions. Their agonists due to neuroprotective and anti-inflammatory effects are considered promising agents for the treatment of epilepsy. However, the changes in PPARs gene expression during epileptogenesis remain poorly studied. In this work, the dynamics of different types of PPARs gene expression in the rat brain in the lithium-pilocarpine (PC) model were studied by RT-qPCR and Western blotting methods. The most pronounced disturbances were found for the PPAR alpha gene: a decrease in its expression was observed in the latent (3 and 7 days after PC injections) and chronic (60 days after PC injections) phases of the model in the ventral and dorsal hippocampus (VH, DH), medial prefrontal and temporal cortex (mPFC, TC). Reduced expression of the PPAR gamma gene was detected in mPFC during the chronic phase and in DH at different test periods. Changes in PPAR beta/delta gene expression were wave-like: an increase in its mRNA production in VH in the latent phase was replaced by a decrease in mPFC in the chronic phase of the model. The decrease in gene expression of these receptors may indicate the suppression of natural protective mechanisms during epileptogenesis. It was also shown that the expression of PPAR-gamma gene in VH can be enhanced by a 30-day treatment with the probiotic Bifidobacterium longum. The findings can be used in the development of a new approach for epilepsy treatment.

Supported by RFBR (N 20-515-00020) and BRFFI (N M20R-328).

THE EFFECT OF AGRP 25-51 ON THE BIOSYNTHESIS OF DOPAMINE AND NOREPINEPHRINE IN THE BRAIN

Mikhrina A.L., Romanova I.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

nastya_mihrina@mail.ru

In the mammalian brain AgRP (agouti gene related protein) is expressed in the neurons of the hypothalamic arcuate nucleus (ARC). During processing three fragments (25-51, 54-82, 83-132) are formed from the AgRP-precursor. The role of AgRP83-132 in the brain is associated with the blockade of GPCR melanocortin receptors 3 and 4. The functions of other AgRP-fragments are not known, but it has been shown that their functions is not associated with GPCR. Processes of AgRP-neurons have been identified in the ventral tegmental area (VTA) and locus coeruleus (LC), where dopamine- and norepinephrine-ergic neurons are located. Was shown that the injections of AgRP83-132 into these brain areas in C57BL/6J mice leads to a decrease in the neurons the level and phosphorylation of tyrosine hydroxylase (TH), a key enzyme in the biosynthesis of catecholamines, as well as a decrease in the level of dopamine (DA) and norepinephrine (HE) in the striatum, where the processes from the VTA and LC come. The aim of this study was to evaluate the systems of DA and NE biosynthetic in the VTA and LC after injection of 0.6 nmol AgRP25-51 into these mouse brain areas. After injections of AgRP 25-51 immunohistochemically in brain sections showed no changes in the level of phospho(serine-40)TH in VTA and LC neurons. However, they showed a decrease in the level of phospho (serine-31)TH and in LC-neurons a decrease in the level of dopamine-beta-hydroxylase. By real-time PCR in the LC region a decrease in the mRNA level of the HE-membrane transporter was shown. The results of HPLC show a decrease in the level of DA and NE in the striatum. The data obtained indicate the inhibitory effect AgRP25-51on the DA- and HE- brain neurons, which is not associated with an effect on the GPCR.

The study was supported by state budget.

THE EFFECT OF MATERNAL HYPERHOMOCYSTEINEMIA ON THE NERVOUS SYSTEM DEVELOPMENT OF RAT FETUS

Shcherbitskaia A.D.[1,2], Milyutina Yu.P.[1], Vasilev D.S.[2], Zalozniaia I.V.[1], Arutjunyan A.V.[1]

1 - Ott Institute of Obstetrics, Gynecology, and Reproductive medicine, St. Petersburg, Russia
2 - Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

nastusiq@gmail.com

Moderate hyperhomocysteinemia (HHC) is a known risk factor for neurodegenerative diseases in humans. During pregnancy, it also leads to various complications in both maternal and fetal organism increasing probability of abnormal brain development and functioning in later life. However, little is known about the mechanisms of homocysteine action on the fetal brain development. Hence, in this study we examined the effects of maternal HHC on neurotrophic factors, apoptosis and cell migration in the fetal brain. Maternal HHC was induced by *per os* administration to Wistar female rats of 0.15% aqueous L-methionine solution (0.10-0.15 g per animal) during pregnancy. According to our data, prenatal HHC led to a decrease in the mBDNF level in the fetal brain on E14 with no effect on E20. Maternal HHC affected the activity of matrix metalloproteinase that regulate processing of neurotrophins. During the fetal brain development, we observed elevated proBDNF content which have the opposite effect on the survival and proliferation of neurons compared to mature forms. The activation of caspase-3 in the brain of fetuses subjected to prenatal HHC was also observed. Structurally, prenatal HHC decreased the total number of labeled cells in the parietal cortex of newborn pups but increased the number of labeled neurons scattered within the superficial cortical layers, suggesting disruption in neuroblast migration during brain development. In addition, changes were found in the expression of semaphorins, which are also key regulators of cell migration, cell death or synapse formation during nervous system development. The data obtained indicate that maternal HHC affect fetal brain development through impaired migration and nerve cells death, as well as disrupted processing of BDNF.

Supported by RFBR 20-015-00388 and Russian state budget assignments 1021062812133-0-3.2.2 and 075-00408-21-00.

THE EFFECTS OF DOPAMINE AND THE DOPAMINE RECEPTOR AGONISTS ON THE PHOTOTRANSDUCTION CASCADE OF FROG RODS

Nikolaeva D.A., Astakhova L.A., Firsov M.L.

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia

daryakorenyak@gmail.com

Dopamine is an important player in retinal physiology. Growing data set provides evidence supporting the role of dopamine as a crucial neuromodulator for light adaptation. It is assumed that in photoreceptors dopamine acts through D_2-like receptors and cAMP/PKA signaling pathway. However, the mechanisms of dopamine-induced effects on the phototransduction cascade have remained unclear. To gain insight into these mechanisms we investigated the effects of dopamine itself and the dopamine receptor agonists on rod photoresponses.

The study was conducted on isolated rods of the frog *Rana ridibunda*. Photoreceptor currents were recorded using a suction pipette technique. The following drugs were tested: dopamine (0.1–50 μM), D_1R agonist SKF-38393 (0.1–50 μM), D_2R agonist quinpirole (2.5–50 μM) and D_1–D_2 receptor heterodimer agonist SKF-83959 (50 μM).

We found that the application of dopamine and the agonists on rod inner segments had no effects on photoresponses. Contrariwise, photoreceptors responded to dopamine, and all agonists applied to rod outer segments by decreasing light sensitivity. These drugs' decreasing order of effectiveness was assessed as SKF-38393 > dopamine > SKF-83959 > quinpirole. The decrease of sensitivity correlates much better with the decrease of the activation rate rather than speeding up the turn-off of the cascade. Moreover, SKF-83959 on average increased the intracellular calcium level, judged from the “exchange current”, by ~1.3 times; while dopamine, SKF-38393, and quinpirole did not change it. Taken together, lack of selectivity and the unidirectional effects of dopamine and the dopamine receptor agonists on light sensitivity supports the assumption that dopamine affects through D_1–D_2 receptor heterodimers and exercises its effects via at least two independent mechanisms mediated by cAMP and Ca^{2+}.

This work was supported by governmental program 075007761902 and in part by RFBR grant 140400428 to MF.

THE EVALUATION OF AUTOPHAGY-RELATED PROTEINS AND GENES IN THE HIPPOCAMPUS AND NEOCORTEX OF RATS

Churilova A., Zachepilo T.

Pavlov Institute of Physiology of Russian Academy of Sciences, St. Petersburg, Russia

annch05@mail.ru

The aim of the study was to estimate the activity of autophagy at rest conditions (in control animals) and after exposure to severe hypobaric hypoxia (SHH, 180 mm Hg, 3 h) in different brain regions (hippocampus and neocortex) of rats. For this, the content of protein markers of autophagy, such as LC3, p62 and LAMP2 and the expression of genes encoding LC3, LAMP2, and TFEB, a transcription factor that regulates transcription of autophagy-related proteins, were assessed. It was revealed that under rest conditions, autophagy activity was higher in the CA1 field of hippocampus in comparison to the V layer of neocortex, - as could be estimated by the level of accumulation of protein markers LC3, p62, LAMP2 after chloroquine injections. SHH led to a decrease in the content of LC3, p62, and LAMP2 1 day after the exposure, but 3 days after SHH the protein levels were restored to control values. Together with this, the expression of *maplc3*, *tf-eb*, and *lamp2* was increased by the 3rd day after SHH in rat hippocampus. In neocortex, no changes in the content of LC3, p62, and LAMP2 were observed after SHH at any time point. The expression of genes associated with macroautophagy, *maplc3* and *tf-eb,* was unchanged, while the expression of *lamp2*, encoding the receptor for chaperone-mediated autophagy LAMP2, was increased by the 3rd hour after SHH. The data obtained indicate that the autophagy process (both of macroautophagy and chaperon-mediated autophagy) is different in the neurons of hippocampus and neocortex of rats. In hippocampus SHH enhanced autophagy-dependent degradation which was followed by enhancement of autophagy-related gene expression. The latter could compensate for the reduced protein pool. In neocortex, the protein levels and gene expression pattern were different from that observed in the hippocampus.

Funding: Russian Foundation for Basic Research, grant 19-04-01152.

THE HIPPOCAMPUS AND ITS NICOTINIC ALFA7 RECEPTORS IN THE INTERACTION OF FUNCTIONS WITH DIFFERENT MODALITY

Zakharova E.I.[1], Storozheva Z.I.[2], Proshin A.T.[3], Monakov M.Y.[1], Dudchenko A.M.[1]

1 - Institute of General Pathology and Pathophysiology, Moscow, Russia
2 - Serbsky' National Medical Research Center for Psychiatry and Narcology, Moscow, Russia
3 - P.K. Anokhin Institute of Normal Physiology, Moscow, Russia

zakharova-ei@yandex.ru

Currently, in the hippocampus, the activation of cholinergic projections through α7 nicotinic receptors (α7nAChRs) is being intensively studied as a necessary component in memory mechanisms. Also, the study of neuronal mechanisms of hypoxic preconditioning is becoming more and more in demand, since the hypoxic preconditioning or its pharmacological imitation has a protective effect in hypoxic and ischemic brain pathologies. In the experiments on rats, we injected intraperitoneally a selective antagonist of α7nAChRs methyllycaconitine (MLA) in the Morris water maze learning model and confirmed a necessity of α7nAChRs preservation for the consolidation of spatial contextual memory during its acute administration to intact animals. In biochemical experiments on a model of one-time moderate hypobaric hypoxia (HBH), we found that the hippocampus and its cholinergic projection system are also key in the mechanism of hypoxic preconditioning to severe hypoxia. The efficiency of HBH is stably associated with acoustic startle prepulse inhibition (PPI) and in rats with PPI ≥ 40%, intraperitoneal and intrahippocampal administration of the α7nAChR agonist PNU-282987 suppressed the efficiency of HBH, while MLA, on the contrary, potentiated it. We assume that under HBH, α7nAChRs are involved in the 'filtering' of cognitive signals and thus are a link in intrahippocampal interaction of functions with different modality. We also believe that a similar mechanism underlie the protective properties of MLA under chronic cerebral hypoperfusion (2VO model). Subchronic administration of MLA in the first hours and days of hypoperfusion significantly reduced the delayed death of animals, without affecting learning. Note that in the sham-treated rats in this model, MLA improved the "rapid one-trial memory", and a negative effect on memory consolidation was less pronounced than with the acute administration of inhibitor. Thus, the evidence is presented that in the hippocampus, the same neuronal elements can perform different roles depending on the modality of exposure.

THE INCREASE OF BLOOD CORTICOSTERONE LEVEL IN ACUTE PERIOD OF TBI IN RATS AS A PREDICTOR OF REMOTE MORTALITY

Shirobokova N.I.[1], Komoltsev I.G.[1,2], Salyp O.Yu.[1], Volkova A.A.[1], Bashkatova D.A.[1], Frankevich S.O.[1,2], Shalneva D.V.[1], Kostyunina O.V.[1], Chizhova O.[1], Kostrukov P.A.[1], Novikova M.R.[1], Gulyaeva N.V.[1,2]

1 - Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia
2 - Moscow Research and Clinical Center for Neuropsychiatry, Moscow, Russia

my.perfect.rat@gmail.com

Introduction: Corticosterone (CS) plays a key role in pathophysiology of late post-traumatic sequelae and potentially may serve as a predictor of mortality. This study was aimed to assess the time course of blood CS after TBI, analyze its contribution to mortality rate and assess the stress reactivity in animals 3 month after TBI.

Methods: Experiment was performed 43 male Sprague-Dawley rats. Animals were divided into 3 groups: TBI, n=22; Sham operated, n=12; control group, n=9. TBI was modeled using lateral fluid percussion. Blood CS level was measured using ELISA one week before craniotomy, in acute (3,7 day after TBI) and late (1, 2, 3 month after TBI) post-traumatic periods. In order to estimate stress reactivity 3 month after craniotomy we measured CS level 30 min after Porsolt test.

Results: Mortality in rats in the acute period of TBI was 22.7% (5 rats), cumulative mortality by the end of the second month 59% (13 rats). Blood CS level increased in the TBI group on day 3 after the injury ($p=0.007$). No other differences between groups in basal CS levels could be detected. Animals which died during the first month after TBI had higher blood CS level on day 3 after the injury as compared to TBI survivors ($p=0.011$). Survival rate was lower in rats with CS>860nmol/l ($p<0.001$, Kaplan-Meier method). CS level 30 minutes after Porsolt test increased in all rats 3 months after craniotomy but and tended to be higher in controls ($p=0.09$).

Conclusion: Craniotomy and TBI induce increase of blood CS level on day 3 after injury and affect stress reactivity 3 month after trauma. CS level higher than in sham rats predicts remote mortality.

Supported by RSF, grant № 22-25-00713

THE ROLE OF ASTROCYTES IN DISTURBANCES OF CALCIUM ACTIVITY OF NERVE CELLS IN MODELING OF ALZHEIMER'S DISEASE IN VITRO

Mitroshina E.V., Yarkov R.S., Krivonosov M.I., Vedunova M.V.

Lobachevsky State University of Nizhny Novgorod, Nizhny Novgorod, Russia

helenmitroshina@gmail.com

The study of the pathogenetic mechanisms of Alzheimer's disease (AD) are among the most pressing topics in neurobiology and medicine. In recent years, there has been more and more information about the role of astrocytes in the pathogenesis of AD. Synaptic transmission and the functioning of neural networks in the development of neurodegenerative processes can be directly modulated by reactive astrocytes. Therefore, our study aimed to investigate the features of the collective dynamics of calcium activity in primary astrocyte cultures derived from the 5xFAD transgenic mouse line (model of familial form of AD), as well as a study of the effect of such altered astrocytes on neural network calcium activity when they are co-cultivated with healthy nerve cells. Primary monoastrocytic cultures were obtained from the cerebral cortex of P1-P2 mice. Astrocyte cultures derived from wild-type mice were used as controls. At 21 DIV, dissociated hippocampal nerve cells from wild-type mice were transplanted to monoastrocytic cultures. Calcium imaging was carried out at 35 DIV (14 DIV of co-cultivation) with Oregon Green 488 (ThermoFisher, USA) used as a calcium sensor. The original software developed by the authors was used to analyze the imaging data (Mitroshina et al. // IJMS, 2020). Astrocyte cultures derived from 5xFAD mice were shown to decrease the degree of correlation of calcium dynamics in neighboring cells (Control – 0.15±0.02; 5xFAD – 0.09±0.01), but not in distantly located cells (Control – 0.09±0.01; 5xFAD – 0.07±0.01). The share of cells exhibiting calcium activity did not change. Astrocytes obtained from 5xFAD mice were found to have a depressing effect on the calcium activity of healthy co-cultured nerve cells. There was a decrease in the level of correlation of calcium activity in both adjacent and distantly located cells, a decline in the number of functional connections per cell, and a drop in the frequency of calcium oscillations' generation.

The study was supported by the Ministry of Science and Higher Education of the Russian Federation (project No № 0729-2020-0061).

THE ROLE OF SODIUM/CALCIUM EXCHANGERS IN THE REGULATION OF NMDA RECEPTOR DESENSITIZATION AND PHARMACOLOGY

Antonov S.M., Sibarov D.A.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

antonov452002@yahoo.com

A fundamental property of ligand-gated ion channels is their desensitization. Many of them, including NMDA-subtype glutamate receptors and voltage-gated ion channels, are characterized by Ca^{2+}-dependent desensitization. It develops due to Ca^{2+}, entering the cytoplasm through open channels, binding to calmodulin, which in this form interacts with the intracellular C-terminal domain of GluN1 subunits of NMDA receptors. This causes a disconnection of receptors with the alpha-actin (cytoskeleton) and provokes the receptor switch to the closed state. Ca^{2+}-dependent desensitization is manifested in the amplitude decrease of NMDA receptor-mediated currents. Since these current are in charge of the glutamate neurotoxic effects, the question arises: "If the enhancement of NMDA-receptor desensitization could counteract the neurotoxic conditions?" We recently discovered that Li^{+}, which by itself do not affect single NMDA receptor channel currents, causes a three-fold drop of amplitudes of NMDA elicited currents and Ca^{2+} responses of neurons. The Na/Ca-exchange inhibitor KB-R7943 also significantly decreases the amplitude of NMDA-activated currents and Ca^{2+}-responses. These observations,disclose a role for the exchanger in the regulation of near-membrane Ca^{2+} concentration and accordingly Ca^{2+}-dependent desensitization of NMDA receptors. Functional interaction between NMDA receptors and Na/Ca-exchanger molecules requires their co-localization in cholesterol-rich membrane microdomains or "rafts." Notably this mechanism may be a target for pharmacological regulation in counteracting the neurotoxic effects of glutamate and homocysteine. Indeed, the inhibitory effect of the tricyclic antidepressants amitriptyline and desipramine (Stepanenko et al., Sci. Rep., 2019; Stepanenko et al., Front. Pharmacol., 2022) on NMDA receptors appeared to be Ca^{2+}-dependent due to provoking of the Ca^{2+}-dependent desensitization. Thus, we discovered a new pharmacological mechanism of action on NMDA receptors - enhancement of their Ca^{2+}-dependent desensitization through influence on Na/Ca-exchange activity.

Supported by the Russian Science Foundation Grant No. 21-15-00403.

THE SEARCH FOR MOLECULAR PHOTOSWITCHES OF NMDA RECEPTORS AMONG AZOBENZENE-CONTAINING QUATERNARY AMMONIUM COMPOUNDS

Nikolaev M.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry Russian Academy of Sciences, St. Petersburg, Russia

fmedfstud@gmail.com

Glutamate ionotropic NMDA receptors play an important role in synaptic plasticity, learning and memory, and are also involved in various pathophysiological processes. The development of light-controlled NMDA receptor modulators - molecular photoswitches - is an important task of modern neuropharmacology. Such substances can help to study the functions of NMDA receptors in the nervous system with high temporal and spatial resolution. We have studied the effect of azobenzene-containing derivatives of quaternary ammonium on NMDA receptors (DENAQ and its analogues). In experiments on native rat hippocampal neurons, we have found that, when applied extracellularly, these substances inhibit transmembrane currents through NMDA receptors. When exposed to monochromatic light, the activity of substances decreases sharply. The action of substances develops quickly and is completely reversible and is not related to the ion channel block. We have discovered a complex molecular mechanism of action consisting of rapid inhibition and slow potentiation. However, the binding site has not been determined and requires additional experiments.

Our findings extend the list of soluble photoswitches of NMDA receptors and opens up the possibility of future elaboration of a new family of light-controlled antagonists for ionotropic glutamate receptors with improved activity and selectivity.

The study was carried out with the financial support of the RFBR, project number 20-04-00218.

THREE-DAY HINDLIMB UNLOADING IMPAIRS DOPAMINE RECEPTOR-ASSOCIATED SIGNALING IN THE STRIATUM OF MICE

Naumova A.A., Khramtsova A.V., Oleynik E.A., Glazova M.V.

Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

anaumova07@gmail.com

Microgravity is the main factor of spaceflights that provokes various motor disorders. In turn, the important role in the regulation of voluntary movements belongs to the striatum. Striatal

neurons are involved in two main neural circuits of the basal ganglia. The direct pathway stimulates the motor cortex and movements, while the indirect one downregulates locomotor activity. A few data suggest that microgravity strongly affects dopaminergic regulation of the striatum, however, mechanisms are poorly understood.

The aim of the present work was to analyze the short-term (3-day) effect of simulated microgravity (hindlimb unloading, HU) on dopamine-dependent signaling in the striatum of mice. Dopamine which regulates striatal neurons is produced by tyrosine hydroxylase (TH) localized in the axon terminals of the substantia nigra. We showed that after 3-day HU TH expression was reduced. However, TH phosphorylation at Ser31 which increases the enzyme activity was higher than in the control. Probably, such up-regulation may compensate decreased TH expression and restore proper dopamine synthesis.

Dopamine binds to D1- and D2-receptors differentially expressed in the striatal cells of direct and indirect pathways. Our data showed increased expression of D1-receptors, while D2-receptor expression was unchanged. Analysis of signaling cascades associated with both receptor types showed unchanged activity of ERK1/2 and Akt/GSK3b cascade, but decreased activity of PKA and transcriptional factor CREB. Analysis of signaling pathways specific for D2-receptors revealed no changes in the activity of CAMKII. These data suggest that D2-associated signaling processes are not affected by unloading, therefore decreased activity of PKA and CREB can result from reduced dopamine activation of D1-containing cells that is not rescued by increased TH phosphorylation and D1-receptor expression.

Thus, our results demonstrate that short-term simulated microgravity primarily affects striatal neurons of the direct pathway and disturbs up-regulation of movement activity.

This study was supported by the RFBR 20-015-00062.

USING EXOSOMES TO CORRECT MEMORY IN 5XFAD MICE

Zhdanova D.Yu.[1], Poltavtseva R.A.[2], Bobkova N.V.[1]

1 - Institute of Cell Biophysics of the Russian Academy of Sciences, Federal Research Center "Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences", Pushchino, Russia

2 - National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov of the Ministry of Healthcare of Russian Federation, Moscow, Russia

ddzhdanova@mail.ru

To date, there is no effective cure for Alzheimer's disease (AD) [Srivastava et.al., 2021].

Nowadays researchers take attention to the using exosomes secreted by multipotent mesenchymal stromal cells (MMSCs), which have a number of advantages, including nanometer size, the ability to overcome the blood-brain barrier, low immunogenicity and the lack of the ability to replicate [Zhdanova et.al., 2021; Soliman et.al., 2021]. Here, a comparative analysis of the effectiveness of intranasal (i/n) administration of exosomes isolated from MMSCs of Wharton's jelly of the human umbilical cord on the memory of 5XFAD mice, model of the hereditary AD, was carried out at different stages of pathology. It was found that the i/n administration of exosomes (6 times, 2 times a week) restored the spatial memory tested in the Morris water maze, both in young (3-4 months) and in 9-month-old transgenic (Tg) animals to the levels of control non-transgenic mice. At the same time, the memory of young untreated Tg mice was better than in aging Tg animals. The positive effect of exosomes persisted one month after their last administration. Immunohistochemical investigation revealed a significant decrease in the density of amyloid plaques in the cerebral cortex of Tg mice treated with exosomes. The addition of exosomes to the primary hippocampal culture of Tg mice induced an increase in cell survival. The positive effect of exosomes from MMSCs allows to consider them as a therapeutic agent for improving memory both in the initial and late phases of neurodegeneration in the hereditary AD.

The reported study was funded by RSF № 18-15-00392.

YAC128 HUNTINGTON'S DISEASE TRANSGENIC MICE DEVELOP HIPPOCAMPAL-ASSOCIATED COGNITIVE IMPAIRMENTS

Grishina E.D.[1], Kraskovskaya N.A.[1], Erofeev A.I.[1], Pushkareva S.A.[1], Gerasimov E.I.[1], Vlasova O.L.[1], Bezprozvanny I.B.[1,2]

1 - Laboratory of Molecular Neurodegeneration, Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia
2 - Department of Physiology, UT Southwestern Medical Center at Dallas, Dallas, USA

Ilya.Bezprozvanny@UTSouthwestern.edu

Cognitive dysfunction in Huntington's disease (HD) is well established and typically explained by degeneration in the cortex and striatum. However, literature data showed that the hippocampus might also be involved in memory loss observed in HD patients. To study the etiology of hippocampus-associated cognitive impairments, behavioral tests, morphology, and electrophysiological experiments were made. We showed that long-term potentiation (LTP) is impaired in 4- and 6-month-old YAC128 HD mice compared to wild type. In addition, there is a trend towards a decrease in the value of post-mechanical potentiation in 4 years old HD mice model. To access dendritic spine morphology, we crossbreed HD mice and M mice which express a

green fluorescent protein in pyramidal neurons of the hippocampus. Our data demonstrate the initial increase in both the dendritic spines' density and the number of mushroom spines in HD mice at the age of 6 months and then a gradual decrease in the spine's density by 9 months.

Using Morris water maze test, we detected impaired spatial memory on 6 months old YAC mice. By the end of the training sessions, wild-type mice showed better results in platform finding, while HD mice were not able to memorize new information and did not improve their parameters by the end of the training sessions, which correlates well with the decrease in synaptic plasticity observed at this age. We also performed a novel object location test to confirm Morris water maze data. Wild-type mice noticed the rearrangement and showed a preference for the object in a novel location while HD mice interacted with both objects equally. Summing up, we conclude that synaptic disorders in Huntington's disease are systemic and degenerative changes in the hippocampus that affect the development of cognitive dysfunction in this pathology.

Supported by RNF № 19-15-00184.

AUTHORS INDEX