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Sincerely,

Walter DiGiusto, President

Application Briefs

Bibliography

July 2012
Use of Microdialysis for Study of Brain Uptake of γ-Hydroxybutyric Acid in Rats
Roiko, et. al., (2012)
Brain Uptake of the Drug of Abuse γ-Hydroxybutyric Acid in Rats Drug Metab Dispos.

Introduction
γ-Hydroxybutyric acid (GHB) is an endogenous compound and a substrate for the ubiquitous monocarboxylate transporter (MCT) family. GHB is also a drug of abuse due to its sedative/hypnotic and euphoric effects, with overdoses resulting in toxicity and death. The goal of this study was to characterize the distribution of GHB into the brain using in vivo microdialysis and in vitro uptake studies and to determine concentration-effect relationships for GHB in a rat animal model. GHB was administered to rats (400, 600, and 800 mg/kg i.v.), and blood, dialysate, and urine were collected for 6 h post-GHB administration. The GHB plasma and extracellular fluid (ECF) concentration-time profiles revealed that GHB concentrations in ECF closely followed plasma GHB concentrations. GHB partitioning into brain ECF was not significantly different at 400, 600, and 800 mg/kg dose. After 120 min, dose-normalized GHB plasma concentrations from the 400 mg/kg dose were reduced compared with the 600 and 800 mg/kg doses, and this decline was also observed in GHB ECF concentrations at the same time point.

Materials & Methods
Evaluation of GHB Concentration-Time Profiles in Brain Using In Vivo Microdialysis
Microdialysis probes (CMA 11; CMA Microdialysis) were prepared according to manufacturer’s instructions and implanted 24 h before the experiment to allow the BBB to reform after implantation (de Lange et al.,2000). All experiments were conducted in metabolic cages in awake and freely moving rats with ad libitum access to water. The experimental protocol is shown in Fig. 1. On the day of the experiment, microdialysis probes were perfused with aCSF at a rate of 2 µL/min and allowed to stabilize for 2 h. Microdialysate fractions were collected every 20 min. After stabilization, the probe was perfused with 1 g/ml GHB in aCSF for 80 min to calculate in vivo probe recovery of GHB for each rat using the established retrodialysis method (Bouw and Hammarlund-Udenaes, 1998). The retrodialysis period was followed by a washout period in which blank aCSF was perfused for 80 min to remove any residual GHB. No GHB was detected in the final two washout samples; endogenous GHB concentrations are below the detection limits for the LC/MS/MS assay.

Results & Conclusions
GHB Microdialysis in Frontal Cortex
The mean in vivo retrodialysis recovery of GHB using the CMA11 probes was 32.5 7.1% (mean S.D.). The GHB plasma and brain ECF concentration-time profiles increased dose dependently after intravenous administration of 400, 600, and 800 mg/kg GHB (Fig. 1, top (plasma) and bottom (brain ECF)). To further characterize the distribution of GHB into brain ECF, plasma and brain ECF concentrations were normalized for dose. The dose-normalized GHB concentrations in plasma and brain ECF at the first six time points (from 5 min, Cmax, through 120 min) were similar and overlapping for the 400, 600, and 800 mg/kg doses. After 120 min, dose-normalized GHB plasma concentrations from the 400 mg/kg dose were reduced compared with the 600 and 800 mg/kg doses, and this decline was also observed in GHB ECF concentrations at the same time point.

GHB concentration-time profiles in plasma (A) and brain extracellular fluid (B) after intravenous administration of GHB 400 mg/kg (c, dotted line), 600 mg/kg (d, dashed line), or 800 mg/kg (e, solid line). The midpoint time represents the midpoint time for each 20-min fraction collection period. Data are plotted as the mean S.D., n 4/dose.
**In Utero Brain Transfection Using Electroporation**

Heterogeneity in Ventricular Zone Neural Precursors Contributes to Neuronal Fate Diversity in the Postnatal Neocortex


*The Journal of Neuroscience, 30(20):7028-7036*

**Introduction**

The recent discovery of short neural precursors (SNPs) in the murine neocortical ventricular zone (VZ) challenges the widely held view that radial glial cells (RGCs) are the sole occupants of this germinal compartment and suggests that precursor variety is an important factor of brain development. Here, we use in utero electroporation and genetic fate mapping to show that SNPs and RGCs cohabit the VZ but display different cell cycle kinetics and generate phenotypically different progeny. In addition, we find that RGC progeny undergo additional rounds of cell division as intermediate progenitor cells (IPCs), whereas SNP progeny generally produce postmitotic neurons directly from the VZ. By clearly defining SNPs as bona fide VZ residents, separate from both RGCs and IPCs, and uncovering their unique proliferative and lineage properties, these results demonstrate how individual neural precursor groups in the embryonic rodent VZ create diversity in the overlying neocortex.

**Materials & Methods**

Female ICR mice were checked daily for vaginal plugs; the day of plug was considered embryonic day 0.5 (E0.5). Surgeries were performed at E13.5 or E14.5. Dams were anesthetized with ketamine/xylazine mixture, and their uterine horns were exposed by midline laparotomy. One microliter of plasmid DNA (3 – 4 µg/µl) mixed with 0.1% fast green dye in phosphate buffer was injected intracerebrally, via pulled micropipette, through the uterine wall and amniotic sac. The anode of the Tweezertrode was placed over the dorsal telencephalon outside the uterine muscle.

Using the ECM 830 & Tweezertrodes 4 pulses of 40 volts and 50 millisecond durations were applied to the target tissue.

**Results & Conclusions**

In utero electroporation preferentially transfects cells that transit through S- and M-phases of the cell cycle within 8 h of the surgery and that the vanguard of the transfected population is in the M-phase at the moment of electroporation.

---

**EXPERIMENT SUMMARIZATION**

Temporal properties of in utero electroporation. BrdU was administered immediately after IUE with pCAG-RFP to label cells in S-phase at the moment of IUE (0 h) (Ai). Using this paradigm, the majority of cells transfected by IUE (RFP) were colabeled with BrdU (70.87±6.60%) (D). Administering BrdU 2 h before IUE labeled cells throughout S- to M-phase at the moment of IUE (2h) (Aii). Nearly the entire RFPpopulation also expressed BrdU using this protocol (95.97±4.00%) (B, B, D). The arrow in B and B denotes an RFPcell that was not BrdU. These data demonstrate that IUE preferentially transfects cells that are in S- and M-phases at the time of electroporation. C, C, Only a small percentage (23.29±4.16%) of cells were colabeled with RFP and BrdU when BrdU was injected 6 h after IUE; the arrows point to the minority of cells that were colabeled. D. The percentage of colabeled BrdU RFPcells decreased as the time between IUE and BrdU administration increased, indicating that plasmid viability is limited.

---

**Products from BTX used in this study:**

Platinum Tweezertrodes

ECM 830 Generator

Part # Description

45-0052 ECM 830 Square Wave Generator

45-0487 Platinum Tweezertrodes (5 mm)

www.btxonline.com
Temperature-Dependent Cysteine Reactivity in the Cystic Fibrosis Transmembrane Conductance Regulator

Introduction

Cysteine scanning has been widely used to identify pore-lining residues in mammalian ion channels, including the cystic fibrosis transmembrane conductance regulator (CFTR). Studies of this type, however, have been typically conducted at room temperature rather than at human body temperature. Reports of substantial effects of temperature on gating and anion conduction in CFTR channels, as well as an unexpected pattern of cysteine reactivity in the sixth transmembrane segment (TM6), prompted the researchers to investigate the effect of temperature on the reactivity of cysteines engineered into TM6 of CFTR. They compared reaction rates at 22 °C or 37 °C in the presence of the mixed disulfide. Increasing the temperature to 32 °C, followed by increasing the superfusate temperature to 32 or 37 °C in the presence of the mixed disulfide, increased the superfusate temperature in the presence of 1 mM extracellular MTSES−-resulted in a decrease in conductance. These results provide evidence of a striking temperature dependence of the reactivity of cysteines engineered into the sixth transmembrane domain (TM6) of the CFTR chloride channel toward a channel-impermeant, thiol-directed reagent. To the best of the researcher’s knowledge, this is the first report comparing substituted cysteines at position 337 of the physiological temperature of 37 °C. The findings have implications for assays routinely used to study the channel-permeant probe 

Materials & Methods

Whole Cell Recordings

Individual oocytes were placed in a 200 mL recording chamber (RC-1Z, Warner Instruments) and continuously perfused with frog Ringer’s solution (4 mL/min). The Ringer’s solution contained 98 mM NaCl, 1 mM MgCl2, 1.8 mM CaCl2, and 5 mM HEPES (pH 7.4). CFTR channels were activated using 10 mM isoproterenol (a β-adrenergic agonist) and 1 mM BMX (a phosphodiesterase inhibitor) as the stimulating cocktail (100 μM-BMX). The solution heater/cooler (Warner Instruments) and the pClamp 8 data acquisition program (Axon Instruments) were used for whole cell recordings. Oocytes were maintained in the open circuit condition, and the membrane potential was periodically ramped from −120 to 60 mV over 1.8 s to construct the whole cell I-V plots.

Temperature Control

A dual automatic temperature controller (CL-200, Warner Instruments) and an in-line solution heater/cooler (SC-20, Warner Instruments) were used to apply acute temperature changes under a constant flow. The bath temperature was monitored. The temperature of the superfusate was returned to 22 °C. Whole cell recordings. Oocytes were maintained in the open circuit condition, and the membrane potential was periodically ramped from −120 to 60 mV over 1.8 s to construct the whole cell I-V plots.

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EXPERIMENT SUMMARY

Materials & Methods

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Results & Conclusions

Representative experiments are compiled in Figure 1. These experiments illustrate the dramatic effect of increased temperature on the rate of reaction of MTSES− with F337C CFTR. In a previous study, we reported that the channel permeant probe [Au(CN)2−] reacted with a cysteine substituted at position 337 while the channel-impermeant probe MTSES− did not. In many such experiments conducted at room temperature, no change in conductance was detectable upon exposure of an oocyte expressing F337C CFTR to 1 mM MTSES−, even for periods exceeding 10 min. Figure 1A depicts exposure of an oocyte expressing F337C CFTR to MTSES− at room temperature. This evoked what at first appeared to be a very slow rate of reaction, but the decline was spontaneously reversed upon removal of the reagent from the superfusate. Subsequent exposure of the oocyte to the channel-permeant probe, [Au(CN)2−], produced a profound inhibition as previously reported, confirming that the cysteine thiolate remained unreacted. Figures 1B and 1C depict experiments in which an oocyte expressing F337C CFTR was exposed to 1 mM MTSES− at 22 °C, followed by increasing the superfusate temperature to 32 or 37 °C in the presence of the mixed disulfide. Increasing the superfusate temperature in the presence of 1 mM extracellular MTSES− resulted in a decrease in conductance. Figure 1D depicts an experiment designed to test the reversibility of the temperature-induced changes in the reactivity of a cysteine at position 337. An oocyte expressing F337C CFTR was heated to 37 °C for 10 min and then cooled to 22 °C. The temperature excursion evoked an increase in conductance that was sustained engineered intact during the period of elevated temperature but was reversed rapidly when the temperature of the superfusate was returned to 22 °C.

Conclusions

These results provide evidence of a striking temperature dependence of the reactivity of cysteines engineered into the sixth transmembrane domain (TM6) of the CFTR chloride channel toward a channel-impermeant, thiol-directed reagent. To the best of the researcher’s knowledge, this is the first report comparing substituted cysteine reactivity at different temperatures, in particular, at the “physiological temperature” of 37 °C. These findings have implications for assays routinely used to test molecular models of the CFTR channel, as well as for the understanding of what structure of the pore domain is most relevant to the function of the channel in vivo.

Products from Warner Instruments used in this study:

- Part # Description Model
  - 64-0318 OC-1Z Oocyte Recording Chamber RC-1Z
  - 64-0028 OC-725C Oocyte Clamp Amplifier OC-725C
  - 64-723 CL-200A Dual Channel Bipolar Temperature Controller CL-200A
  - 64-0355 SC-20 Solution Heater/Cooler, Dual Channel

www.warneronline.com
Chronic Cocaine Self-Administration Modulates ERK1/2 & CREB Responses to Dopamine Receptor Agonists in Striatal Slices


Introduction
Self-administration is a classical model of human drug-taking behavior and consists in establishing in rodents an operant conditioning paradigm. Self-administration paradigm is used in rat for assessing the effect of chronic cocaine self-administration on the expression of the extracellular signal-regulated protein kinase 1 and 2 (ERK1/2) and the cyclic adenosine monophosphate response element-binding protein (CREB) phosphorylation related with the activation of D1 and D2 dopamine receptors in the Nucleus Accumbens (Nac) and Striatum.

Materials & Methods
Self-administration procedure has been used in male Sprague–Dawley-OFA rats. Rats were tested in standard operant conditioning chambers (25-cm-wide x 25-cm-high x 25-cm-deep) located inside sound-attenuating cubicles with a ventilating fan. Two metal response levers were located on either side of the food tray. Placed above each lever was a round disc (4-cm diameter) that could be illuminated by a 2.4-W, 24-V light bulb, which served as a stimulus light. The rear panel of the chamber contained an attachment for a water bottle. Intravenous infusions of cocaine were administered by a syringe pump placed above the sound attenuating box through a counterbalanced single channel liquidswivel. The lead that delivered the drug solution to the animal was flexible and wrapped with chew-resistant wire. This tubing could be connected to a 24-gauge guide cannula attached to the animal-implanted catheter. Behavioral equipment and data collection were controlled by the Packwin Software.

The self-administration procedure was divided in three different steps:

Sucrose Training: The animals are food-deprived and trained to press a lever for receiving sucrose pellets. A first initial overnight sucrose-training session is carried out during the dark cycle. The animal is considered as trained when it successfully obtains 100 sucrose pellets during two additional 2-hour sessions in the light cycle.

Surgery: The animals are operated for catheter implantation in the right jugular vein for allowing the intravenous administration of the drug. The Nucleus accumbens and striatal slices are then used for ex vivo western blot analysis in order to assess the expression of the ERK1/2 and CREB protein related to the D1 and D2 dopamine receptors.

Results & Conclusions

Results
The authors found that cocaine self-administration led to a reduction in the capacity of D1R to activate ERK1/2/phosphorylation as compared with control rats.

Altered agonist-induced signaling was independent of total ERK1/2 and CREB expression.

Conclusions
In conclusion, the results of this study suggest that chronic cocaine self-administration induces changes in the signaling strength of D1R and D2R, leading to decreased D1R responses and increased D2R-mediated CREB phosphorylation in the NAc. The finding that selected cellular D2R responses to CREB were strengthened by cocaine self-administration could be relevant to understand how dopaminergic receptors participate in cocaine-induced behaviors.

EXPERIMENT SUMMARIZATION

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www.panlab.com

Products from Panlab used in this study:
**D-Cycloserine Facilitates Extinction of Cocaine Self-Administration in Rats**


**Introduction**

Cocaine increases dopamine levels by blocking dopamine transporters, thus inducing rewarding effects leading to addiction. AMPA and NMDA receptors have been linked to cocaine-seeking behavior and this study investigates the therapeutic potential of medications targeting these receptors, specifically D-cycloserine (DCS) which as illustrated promise in the extinction of previously conditioned fear and anxiety. A rat model of cocaine self-administration is used to measure voluntary consumption and the motivational drive of cocaine seeking.

Subjects were trained to respond to an operant lever with a food pellet reward. Coulbourn Self-Administration test cages with isolation cubicles were used. Each test cage contained two levers, one considered “active” on the left and one considered “inactive” on the right, both with cue lights above the levers. A pellet receptacle was located between the two levers. An IR activity monitor was located on the test cage back wall to monitor locomotor activity. Using a fixed-ratio 1 (FR1) reinforcement schedule with a 30 second timeout, animals were trained with 90 minute sessions per day for a period of four-days. During the 30 second timeout period, food was not released, but responses recorded. Inactive lever presses offered no consequence.

Once successful lever discrimination was achieved, subjects underwent catheterization surgery. Indwelling catheters were implanted in the right jugular vein to allow for intra-venous cocaine self-administration. Following recovery, each animal again was tested to ensure a conditioned response criterion was achieved. Cocaine self-administration sessions for 90 minutes per day over a 15 day period were conducted in the dark cycle, again using a FR1 schedule with 30 second timeout period. At the start of each session, subjects received a priming dose of the cocaine (0.75 mg/kg/infusion, i.v.). A single press on the “active” lever provided immediate delivery of the same dose with a 30 second timeout, during which the cue light above the active lever was activated and drug was no longer available. Lever presses during the timeout period counted as were presses on the “inactive” lever, neither of which had consequences. Initial cocaine doses were 0.75 mg/kg/infusion in a volume of 0.1 ml for the first seven days, and the dose was halved to 0.375 mg/kg/infusion for the last eight days.

Following the self-administration phase, rats were randomly assigned to one of three groups; control, low dose DCS (15 mg/kg i.p. DCS) or high does DCS (30 mg/kg i.p. DCS). Extraction procedures followed that of the self-administration period, 90 min/day sessions for 15 days where a single lever press of the “active” lever had an immediate delivery of saline. After each extinction session, the subjects were injected with the control, 15 mg/kg or 30 mg/kg i.p. DCS depending on their assigned treatment group.

**Results and Conclusions**

Food training allowed the subjects to differentiate between the “active” and “inactive” levers. A significantly greater number of “active” lever presses compared to “inactive” presses was maintained throughout the 15-days of the cocaine self-administration. In addition, a significantly greater number of lever presses are noted on days 12-19 when the cocaine concentration was halved to 0.375 mg/kg/infusion.

Data from the extinction period illustrated a significant difference between all three treatment groups in the number of “active” lever presses. In addition, there was a significant variation in the number of days for subjects in the different groups to achieve extinction. The higher dose of DCS, 30 mg/kg i.p. treatment illustrated a significantly faster rate of extinguishing “active” lever responses.

Activity monitoring showed an expected increase in locomotor activity during the cocaine self-administration phase, compared with the food training. Activity in the extinction phase was consistent among the three treatment groups.

Investigators concluded that DCS may have a therapeutic value for the extinction of cocaine seeking behavior, noting a dosing effect.
Microdialysis Probes into Rat Blood & Brain

X. Zhou et al. (2011)
J. Chromatogr., B 879 3041–3046

Introduction
To evaluate the penetration of the blood–brain barrier by 9-fluoropropyl(+)-dihydrotetrabenazine (AV-133), microdialysis probes were implanted simultaneously into rat blood and brain, and a liquid chromatography–tandem mass spectrometric method was developed and validated to monitor the AV-133 concentration in the microdialysates. This method was used to determine the concentrations of AV-133 and its pharmacokinetics in the brains and blood of rats.

Materials & Methods

Recovery of the Microdialysate
The relative recovery of AV-133 with the microdialysis probe was estimated in delivery experiments. Perfusion solutions containing AV-133 (100 ng/mL) were passed through the microdialysis probes and separately into the rat blood and brain at a constant flow rate of 1 μL/min. One hour after probe implantation, the perfusate (Cperf) and dialysate (Cdial) concentrations of AV-133 were determined by LC–MS/MS. The in vivo relative recovery (Rdial) of AV-133 across the microdialysis probe was calculated with the following equation:

\[ \text{Rdial} = \frac{\text{Cperf} - \text{Cdial}}{\text{Cperf}}. \]

Implantation of the Microdialysis Probe
The simultaneous collection of the dialysate from the blood and brain offers a useful approach to monitoring drug concentrations centrally and peripherally during drug administration. Two microdialysis probes were implanted: one into a specific brain region and the other into the jugular vein.

The rats were anesthetized throughout the experiments and a temperature-controlled heating pad was used to maintain their body temperature. The CMA 20 Elite Microdialysis Probe, 10 mm membrane length (CMA Microdialysis), was implanted into the jugular vein toward the rats’ right atrium. An intracerebral guide cannula was implanted into the striatum at the coordinates: AP 0.2 mm, ML −3.2 mm, and DV −7.5 mm, according to the Paxinos and Watson atlas. A hole was drilled for the guide cannula, which was fixed to the skull with two anchor screws and dental cement. A CMA 12 brain microdialysis probe, 4 mm membrane length (CMA Microdialysis) was inserted into the guide cannula.

Results & Conclusions
Relative recoveries of the Microdialysis probes
Based on the delivery experiments, the relative recoveries were determined to be 49.6 ± 2.3% for the plasma vascular probe and 33.4 ± 2.6% for the brain probe. The concentrations of AV-133 determined in the physiological samples were corrected for the relative recovery of the probe used.

Pharmacokinetics and Data Analysis
The concentration versus time curves for AV-133 in anesthetized rat blood and brain after the administration of AV-133 (5 mg/kg, i.v.) are shown in Fig. 3. The peak concentration (Cmax) of AV-133 in the blood was 655.53 ng/mL, whereas AV-133 reached a peak concentration of 1062.95 ng/mL in the brain at 15 min, which suggests that AV-133 is quickly distributed to the brain.

The blood-to-brain distribution ratio was defined by dividing the area under the concentration versus time curve (AUC) for the brain by that for the blood (AUCbrain/AUCblood) [20]. The value was 1.03, which indicates that AV-133 readily crossed the BBB, and the degree of drug penetration into the brain was high. The appropriate half-life and excellent BBB penetration of AV-133 are highly favorable characteristics for a PET imaging agent.

Fig. 1. Plasma concentration–time curves for AV-133 in rat blood (−−→) and in the rat striatum (——) after the i.v. administration of AV-133 (5 mg/kg).

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www.microdialysis.com
Effects of Chronic Alcohol Treatment on Acoustic Startle Reactivity During Withdrawal & Subsequent Alcohol Intake in High & Low Alcohol Drinking Rats

Julia A. Chester, Annette M. Blose and Janice C. Froehlich (2005)
Alcohol and Alcoholism, 40(5), 379-387

Introduction
Chronic alcohol drinking leads to a number of neurobiological processes, most notable alcohol dependence. Alcohol withdrawal syndrome emerges from the dependence with both physical signs and subjective symptoms. In the present work, the authors seek to investigate the effect of the alcohol withdrawal experience as a deterrent to alcohol consumption in both high and low alcohol drinking subjects.

Materials & Methods
The experiment was performed on alcohol-naïve adult male rats, selectively bred for alcohol preference [HAD2 (high alcohol drinking) and LAD (low alcohol drinking)] within an acoustic startle chamber, consisting of four weight sensitive platforms inside an isolation cubicule.

During a 3-day acclimation period, the subjects were introduced to the handling, infusions, and acoustic startle equipment to reduce the impact of stress. On the first day, the subjects were handled in their colony room by the experimenter and an oral gavage with metal feeding tube was inserted, although no treatment was given. On the second day, animals were transported on a cart to the experiment room, handled, given another oral gavage without treatment, and placed in the acoustic startle system for 15 minutes with only background noise audible. On the last day of the habituation period, all subjects were again transported, gavaged without treatment and placed within the startle chamber. A baseline session was conducted for 15 minutes where the subjects were exposed to the acoustic tone stimuli that would be used in the experimental session, 90, 100, 110, and 120 dB.

Following the acclimation period, the subjects were grouped within each line based on body weight and assigned in matched pairs to receive either alcohol or water treatment. Each subject received two infusions or alcohol (3.0 g/kg BW; 25% v/v) or an equal volume of water at 5 hour intervals for a period of 20 consecutive days. Acoustic startle response was measured 10, 14, and 18 hours after the second infusion on days 1, 5, 10, 15 and 20 to best assess alcohol withdrawal after the chronic exposure.

BAC analysis was performed on all subjects to correlate BAC to times of alcohol withdrawal testing and to identify any change in alcohol metabolism in the two lines over the testing period.

Acoustic startle response magnitude was determined for each subject as an average of the five highest startle responses (grams of force) to each tone stimulus, with 20g force minimum required for response to be considered a true startle response.

Results and Conclusions
Only startle responses to 120 dB (140 Hz) were reported as lower tones did not produce qualified startle responses in either HAD2 or LAD2 groups.

The mean startle magnitude in response to the 120 dB tone was greater overall for the LAD2 subjects, in both treatment groups, compared to the HAD2 groups, as seen in Figure 1. Startle magnitude decreased for all groups over the progression of test days, indicating suppression due to alcohol withdrawal for both LAD2 and HAD2.

An impact of testing time post-treatment was found on day 10, with a startle magnitude decrease for all groups across the three testing time points for that test day.

This experiment illustrates our Coulbourn’s Acoustic Animal Startle System can be used to investigate the genetic association between alcohol withdrawal severity and alcohol drinking behavior as impacted by prior chronic alcohol exposure.

EXPERIMENT SUMMARIZATION

Materials & Methods
The experiment was performed on alcohol-naïve adult male rats, selectively bred for alcohol preference [HAD2 (high alcohol drinking) and LAD (low alcohol drinking)] within an acoustic startle chamber, consisting of four weight sensitive platforms inside an isolation cubicule.

During a 3-day acclimation period, the subjects were introduced to the handling, infusions, and acoustic startle equipment to reduce the impact of stress. On the first day, the subjects were handled in their colony room by the experimenter and an oral gavage with metal feeding tube was inserted, although no treatment was given. On the second day, animals were transported on a cart to the experiment room, handled, given another oral gavage without treatment, and placed in the acoustic startle system for 15 minutes with only background noise audible. On the last day of the habituation period, all subjects were again transported, gavaged without treatment and placed within the startle chamber. A baseline session was conducted for 15 minutes where the subjects were exposed to the acoustic tone stimuli that would be used in the experimental session, 90, 100, 110, and 120 dB.

Following the acclimation period, the subjects were grouped within each line based on body weight and assigned in matched pairs to receive either alcohol or water treatment. Each subject received two infusions or alcohol (3.0 g/kg BW; 25% v/v) or an equal volume of water at 5 hour intervals for a period of 20 consecutive days. Acoustic startle response was measured 10, 14, and 18 hours after the second infusion on days 1, 5, 10, 15 and 20 to best assess alcohol withdrawal after the chronic exposure.

BAC analysis was performed on all subjects to correlate BAC to times of alcohol withdrawal testing and to identify any change in alcohol metabolism in the two lines over the testing period.

Acoustic startle response magnitude was determined for each subject as an average of the five highest startle responses (grams of force) to each tone stimulus, with 20g force minimum required for response to be considered a true startle response.

Results and Conclusions
Only startle responses to 120 dB (140 Hz) were reported as lower tones did not produce qualified startle responses in either HAD2 or LAD2 groups.

The mean startle magnitude in response to the 120 dB tone was greater overall for the LAD2 subjects, in both treatment groups, compared to the HAD2 groups, as seen in Figure 1. Startle magnitude decreased for all groups over the progression of test days, indicating suppression due to alcohol withdrawal for both LAD2 and HAD2.

An impact of testing time post-treatment was found on day 10, with a startle magnitude decrease for all groups across the three testing time points for that test day.

This experiment illustrates our Coulbourn’s Acoustic Animal Startle System can be used to investigate the genetic association between alcohol withdrawal severity and alcohol drinking behavior as impacted by prior chronic alcohol exposure.

Introduction
Chronic alcohol drinking leads to a number of neurobiological processes, most notable alcohol dependence. Alcohol withdrawal syndrome emerges from the dependence with both physical signs and subjective symptoms. In the present work, the authors seek to investigate the effect of the alcohol withdrawal experience as a deterrent to alcohol consumption in both high and low alcohol drinking subjects.
Novel Indirect Calorimetry Technology to Analyze Metabolism in Individual Neonatal Rodent Pups

Materials & Methods

Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were used in the investigation. The effect of litter size was assessed by comparing data between 3 experimental groups. On P1, one litter was culled to 5 pups (SML), one litter was culled to 8 pups (MED), and one litter was culled to 11 pups (LRG).

Small Plexiglas of 200 ml was used for P2-P10 neonates and of 400 ml for P11-P14  neonates. The chambers were placed on control heating pads at 35º (typical nest temperature), resulting in an ambient temperature of 28-29º in the chamber. The heating pad was placed underneath a 10-gallon aquarium containing a Pyrex pan filled with distilled water. A Plexiglas sheet was placed over the Pyrex pan and the round Plexiglas metabolic chamber containing a small amount of bedding material was placed on it (see next photo).

Individual pups acclimatization in chamber: 10-15 minutes prior to data collection. Data collection paradigm: 3 cycles of 5 mins room air sampling alternating with 20 mins chamber sampling (30 mins of respiratory gas analysis per pup). To minimize stress, the total time the pup spent away from the dam was limited to 1 h.

Air flow for P1-P5 pups: 40-45 ml/min and for P6-P14 pups: 100-125 ml/min. Switching time: 3 min.

The METABOLISM software is used for data storage, compilation and Respiratory Quotient (RQ) calculations.

Results & Conclusions

Data revealed that the only metabolic parameter influenced by litter size is a neonatal rat’s RQ, with rat pups reared in a small litter (5 pups) having lower RQ’s than rat pups reared in either medium (8 pups) or large (11 pups) litters. Furthermore, data showed that ambient temperature affected all metabolic parameters measured, with colder temperatures being associated with higher CO2 production, higher O2 consumption, and higher energy expenditure.

The respiratory quotient RQ gives an indication about the nature of the diet: Normal diet: RQ around 0.8; Fats rich diet: RQ around 0.6; Carbohydrates rich diet: RQ around 1.0.

A litter size of 4 pups is associated with an increased dam milk fat concentration from P6-10 and P10-14 compared to pups in litters of 10 or 16. The small litter size is also associated with significantly lower dam milk protein content from P4-6, P6-10, and P10-14 compared to larger litters.

The findings of a reduced RQ value in the SML group compared to another.

Conclusions

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The findings of a reduced RQ value in the SML group compared to another.
phiC31 Integrase Confers Genomic Integration and Long-Term Transgene Expression in Rat Retina

Reference; Chalberg, T. et al., (2005)
.phiC31 integrase confers genomic integration and long-term transgene expression in rat retina. Invest Ophthalm. Cis. Sci.; 46 (6), 2140-6

Introduction
Gene therapy has shown promise in animal models of retinal disease, with the most success achieved to date with viral vectors used for gene delivery. Viral vectors, however, have side effects and limitations and are difficult to manufacture. The present study was conducted in an attempt to develop a novel system for long-term gene transfer in rat retinal pigment epithelium (RPE), by using nonviral transfection methods for gene transfer and the integrase from the bacteriophage C31 to confer long-term gene expression by means of genomic integration.

Materials & Methods
Efficient nonviral delivery of plasmid DNA to rat RPE in vivo was achieved by using subretinal injection of plasmid DNA, followed by in situ electroporation. Gene delivery was evaluated by analyzing enhanced green fluorescent protein (eGFP) expression in frozen sections. In subsequent experiments, a plasmid expressing luciferase, with or without a plasmid encoding the C31 integrase, was delivered to rat RPE. Luciferase expression was followed over time by using in vivo luciferase imaging.

ECM® 830 In Vivo Electroporation Protocol

TISSUE PREPARATION:
Perform subretinal injections in the superior hemisphere of the animal. Soak BTX Tweezertrodes in PBS and apply to each cornea with negative electrode on the injected eye with 14mm between electrodes. (Figure 2 below)

ELECTROPORATION SETTINGS:
Voltage: 140 V
Pulse Length: 100 msec
Number of Pulses: 5
Interval: 950 ms
Field Strength: 100 V/cm

Results & Conclusions

Results
Subretinal injection followed by electroporation yielded abundant transgene expression in the rat RPE. Expression was strongest 48 hours after delivery. In the absence of C31 integrase, transgene expression declined to near-background levels within 3 to 4 weeks after treatment. By contrast, coinjection of the integrase plasmid led to long-term stable transgene expression throughout the 4.5-month test period. Eyes injected with C31 integrase showed 85-fold higher long-term transgene expression in the retina than eyes without integrase.

Conclusions
Subretinal injection of DNA followed by electroporation affords abundant transfer of plasmid DNA in rat RPE. C31 integrase confers robust long-term transgene expression by mediating genomic integration of the transgene. These findings suggest that C31 integrase may be a simple and effective tool for nonviral long-term gene transfer in the eye.

Arrangement of electrodes for in vivo electroporation for RPE transfection. (A) Tweezer-type electrodes were placed on the corneal surface of either eye of a 1-month-old Sprague-Dawley rat. (B) The current was applied with the positive electrode contralateral to the injected eye. After prior injection of plasmid DNA into the subretinal space of the right eye, this arrangement electrophoresed the negatively-charged DNA toward the RPE layer (arrowheads).
**Temperature Dependence of Ca^{2+} Fluxes in Scombrid Cardiomyocytes**

**Introduction**

Specializations in excitation-contraction (E-C) coupling may have played an important role in the evolution of endothermy and high cardiac performance in scombrid fishes. The researchers examined aspects of Ca^{2+} handling in cardiomyocytes from Pacific bonito (Sarda chilensis), Pacific mackerel (Scomber japonicus), yellowfin tuna (Thunnus albacares) and Pacific bluefin tuna (Thunnus orientalis). The whole-cell voltage-clamp technique was used to measure the temperature sensitivity of the L-type Ca^{2+} channel current (ICa) and density, and steady-state (ssSRload) and maximal (maxSRload) sarcoplasmic reticulum (SR) Ca^{2+} content. These data were acquired with the aid of a PC-505B patch clamp amplifier, a CL-100 bipolar temperature controller, and an SC-20 in-line solution heater/cooler from Warner Instruments.

**Materials & Methods**

**Experimental Procedure**

Voltage clamp experiments were conducted in the whole-cell configuration using a PC-505B amplifier with a 202B headstage (Warner Instruments). Micropipettes were made from borosilicate glass (Garnet Glass) using a P-87 pipette puller (Sutter Instrument) and had resistances of 2-3.5 MΩ when filled with pipette solution. Junction potentials were zeroed prior to seal formation and pipette capacitance was compensated for once a gigohm seal was achieved. Access to the cell was obtained by rupturing the membrane patch with a brief voltage pulse. Capacitive transients were compensated for by adjusting the series resistance and cell capacitance compensation circuits. Mean series resistance was 9.7±0.5 MΩ. Currents were low-pass filtered at a frequency of 2 kHz and analyzed offline using Clampfit 9.2 (Axon Instruments).

**Measurement of ICa**

ICa was activated using the protocol illustrated in Figure 1. Current-voltage relationships were elicited by depolarizing the cell from -80 to +60 mV in steps of 5 mV in 15°C increments. The amplitude of the L-type Ca^{2+} channel was calculated as the difference between the maximal inward current deflection and the current at the end of the depolarizing pulse. ICa was normalized for cell size by dividing ICa by cell capacitance. Current-voltage curves were obtained at 15, 19 and 23°C by adjusting superfusion temperature through a temperature-controlled rapid solution changer (SC-20 In-line Solution Heater/Cooler; Warner Instruments).

**Results & Conclusions**

Characterization of ICa in Scombrid Cardiomyocytes

In all species, a voltage step from -80 to 0 mV in the presence of tetrodotoxin (TTX) gave rise to a slow activating and inactivating ICa that varied in amplitude and kinetics according to temperature (Figure 1A). To confirm that this current originated from the L-type Ca^{2+} channel, nifedipine, a specific L-type Ca^{2+} channel inhibitor, was applied that completely abolished ICa.

**ICa-voltage Relationships and Kinetics**

The temperature dependence of the ICa-voltage relationship for each species tested is shown in Figures 1B-E. Peak ICa amplitude was similar between mackerel and yellowfin tuna at each temperature tested (figures 1B-D).

**Conclusion**

The major findings from this study are (1) the magnitude of ventricular ICa density does not correlate with endothermy, cardiovascular capacity, or thermal niche expansion, (2) SR Ca^{2+} content is greater in the more cold-tolerant species (bluefin tuna and mackerel), and (3) bonito utilizes a different E-C coupling strategy from mackerel and tuna. All scombrid fishes showed significant reductions in ICa with decreasing temperature. In the mackerel and yellowfin, the slowing of inactivation kinetics of ICa offset the negative effects of temperature, resulting in a temperature-insensitive Ca^{2+} flux. In contrast, the Ca^{2+} carried by ICa in the bluefin tuna and mackerel appears to be temperature sensitive with distinctly different affects.

**Experiment Summary**

**ICa-voltage Relationships and Kinetics**

**Fig. 1. Effect of acute temperature changes on L-type Ca^{2+} channel current-voltage relationships in ventricular myocytes from the Pacific mackerel, bonito, yellowfin tuna and Pacific bluefin tuna. (A) Representative trace and voltage protocol. (B–E) The current-voltage relationship for ICa for each scombrid. Red circles, 23°C; dark blue circles, 19°C; light blue circles, 15°C.**

**Products from Warner Instruments used in this study:**

- **PC-505B Patch Clamp**
- **Bipolar Temperature Controller**
- **Solution Heater/Cooler**
- **SC-20**

**Part #** | **Description** | **Model**
--- | --- | ---
64-0001 | PC-505B patch clamp with HC-202B headstage (50 GΩ/50 MΩ) | PC-505BHC
64-0352 | Bipolar temperature controller | CL-100
64-0353 | Solution heater/cooler, dual channel | SC-20

www.warneronline.com
Use of Syringe Pumps for Injection into Brain Structures

Functional Coupling between the Prefrontal Cortex and Dopamine Neurons in the Ventral Tegmental Area,
The Journal of Neuroscience, 27(20): 5414-5421

Introduction
Stimulation of the prefrontal cortex (PFC) has been shown to have an excitatory influence on dopamine (DA) neurons. We report here that, under nonstimulated conditions, the activity of DA neurons in the ventral tegmental area (VTA) also covaries, on a subsecond timescale, with the activity of PFC neurons. Thus, in 67% of VTA DA neurons recorded in chloral hydrate-anesthetized rats, the firing of the cell displayed a slow oscillation (SO) that was highly coherent with the activity of PFC neurons. The SO was suppressed by transections immediately caudal to the PFC or by intra-PFC infusion of tetrodotoxin, suggesting that it depends on inputs derived from the PFC. Unexpectedly, the SO in most VTA DA neurons was reversed in phase relative to PFC cell activity, suggesting that at least part of PFC information is transferred to DA neurons indirectly through inhibitory relay neurons. Further understanding of this coordinated activity may provide important new insights into brain functions and disorders thought to involve both VTA DA and PFC neurons.

Materials & Methods
To test whether PFC inputs are involved in the generation of the SO in DA neurons, bilateral transections were made immediately caudal to the PFC to interrupt PFC inputs to DA neurons. A slit was drilled in the skull 2.0 mm anterior to bregma. The sharp pointed tip of a 3-mm-wide scalp blade was lowered to the base of skull and passed along the slit to completely interrupt connections between the PFC and the rest of the brain. Recordings commenced 30 min after the transection.

In a second group of rats, the PFC was inactivated by local infusion of TTX (Fisheries Science and Technology Development, Hebei, China). Silica capillary tubing (outer diameter, 160 µm; inner diameter, 100 µm) was filled with TTX (10 ng/µl in 0.9% saline) and inserted into the medial PFC (from bregma, AP, 3.0; ML, 0.7; DV, 3.5 mm). After a stable baseline recording, TTX was infused at 0.75 µl/min for 2 min (controlled by a syringe pump; Harvard Apparatus). Effects of TTX infusion were determined by comparing recordings from the same cell before and after the infusion. Only one cell was studied in each rat. In separate groups of animals, saline (1.5 µl) was infused into the mPFC in a similar manner, or TTX (15 ng/1.5 µl) was infused into the lateral PFC (from bregma, AP, 3.0; ML, 1.4; DV, 3.5). At the end of the experiment, the injection site was verified using standard histology methods.

Results & Conclusions
Phase relationship between DA cell firing and VTA LFPs. A, Left, Segments of spike trains recorded from a VTA DA neuron, the corresponding rate histogram, and concurrently recorded LFPs from the VTA and PFC. Right, Cross, coherence, and phase spectra between the firing of the DA cell and VTA LFPs (D→V, top charts) and between VTA and PFC LFPs (V→P, bottom charts). As in most DA neurons, the SO in this DA cell had a nearly antiphase relationship with VTA LFPs. The latter showed a nearly in-phase relationship with PFC LFPs. B, Distributions of phase lags between DA cell firing and VTA LFPs (DA→V), between DA cell firing and PFC LFPs (DA→P), and between VTA and PFC LFPs (V→P). Right, y-axis values are percentages of cells that showed significant coherence with PFC or VTA LFPs.

Products from Harvard Apparatus used in this study:

Harvard Pump 11 Elite

Part # Description
70-4505 Pump 11 Elite Infusion/Withdrawal Programmable Dual Syringe

www.harvardapparatus.com
Effects of Dopaminergic Drugs on Innate Pheromone-Mediated Reward in Female Mice: A New Case of Dopamine-Independent “Liking”


Introduction
Male sexual pheromones are innately rewarding to adult female mice, but the role of dopamine in this natural reward is unknown. In the present work, the authors have tackled this issue by assessing the effects of different substances acting on the dopaminergic system (dopamine-releasing agents, D1 and D2 dopamine receptor agonists and antagonists) on the chemoinvestigation displayed by female mice in male-versus female-soiled bedding 2-choice tests.

Materials & Methods
The experiment was performed on female mice in a rectangular cage, symmetrically divided in two areas where are located a glass dish and external visual cues. After 2 days of habituation to the experimenter and the test cage (10 min/day) with both dishes containing clean bedding, the behavior of the mice was video for 10 min (pretreatment control test), and the time they spent investigating each dish was measured to check whether the exploration of the two sides of the test cage was balanced.

From day 1 to day 5, mice received intraperitoneal injections of saline, SCH or sulpiride (D1 and D2 dopaminergic receptor antagonists respectively), 20 min before the training sessions. Training consisted of a daily 10-min two-choice preference test (male-soiled bedding vs. clean bedding), in which male-soiled bedding was presented systematically either in the right or in the left dish of the cage (‘rewarded area’, opposed to ‘nonrewarded area’ in which the dish contains clean bedding).

At day 6, the conditioned place preference for the location where male pheromones were presented during trainings is tested by observing time spent in exploring the areas, where dishes both contain clean bedding.

Results & Conclusions
The calculation of the time spent by the subjects in a semicircle (20 cm of diameter) around the center of the dishes reveals the interest of the mouse for the dish content.

There are no differences between sides in the control pretreatment tests indicating an unbiased design (1A and 2, Control).

In the place-preference test, females of all groups displayed a significant preference for the zone where male soiled bedding had been located during training sessions (1B and 2, Place preference test), validating this new place conditioning paradigm induced by male pheromones.

Neither D1 nor D2 antagonists affected the induction of conditioned place preference by male sexual pheromones indicating that this effect do not involve the activation of the dopaminergic systems.

EXPERIMENT SUMMARIZATION

Materials & Methods
The experiment was performed on female mice in a rectangular cage, symmetrically divided in two areas where are located a glass dish and external visual cues. After 2 days of habituation to the experimenter and the test cage (10 min/day) with both dishes containing clean bedding, the behavior of the mice was video for 10 min (pretreatment control test), and the time they spent investigating each dish was measured to check whether the exploration of the two sides of the test cage was balanced.

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Neither D1 nor D2 antagonists affected the induction of conditioned place preference by male sexual pheromones indicating that this effect do not involve the activation of the dopaminergic systems.
The Artificial Gene Jazz, a Transcriptional Regulator of Utrophin, Corrects the Dystrophic Pathology in Mdx Mice

Grazia DiCerto, M., Corbi, N., Strimpakos, G., Onori, E.M., Pisani, C., Floridi, A., Benassi, B., Fanciulli, M., Regulator of Utrophin, Jazz, a Transcriptional zinc finger transcription factors (ZF ATFs). The ZF ATF technology for DMD treatment was recently engineered and tested in mice. Here, we show that the artificial Jazz transgenic mice with dystrophin-deficient mdx mice was maintained throughout the life of the animals. The absence of the cytoskeletal protein dystrophin results in Duchenne muscular dystrophy (DMD). The utrophin protein is the best candidate for dystrophin replacement in DMD patients. To obtain therapeutic levels of utrophin expression in dystrophic muscle, we developed an alternative strategy based on the use of artificial zinc finger transcription factors (ZF ATFs). The ZF ATF ‘Jazz’ was recently engineered and tested in vivo by generating a transgenic mouse specifically expressing Jazz at the muscular level. To validate the ZF ATF technology for DMD treatment we generated a second mouse model by crossing mdx mice with dystrophic muscles in vivo. Different groups of mice (WT, mdx and both Jazz-negative and Jazz-positive mdx mice) at 3 or 12 months of age, were subjected to forced physical exercise on an accelerating treadmill. The exercise was repeated once a week for four consecutive weeks and the running time was recorded in each session. Shock intensity was set at 0.4 mA. Inclination of treadmill was set at 0°. During the first session, mice were first familiarized to the treadmill apparatus for 2 min before starting the belt followed by a running with speed set at 6 m/min. Each mouse was immediately removed from treadmill after 3 electric shock. One day after habituation, mice were subjected to a training protocol with treadmill running at mixed speed. Mice were first acclimated to the treadmill apparatus for 2 min, followed by a running with speed initially set at 6 m/min. At 5, 15, 65, 75 min after the initiation of exercise, the speed was increased for 5 min by 0.6 m/min. At the end of this incremental procedure, the final speed was 18 m/min. Exercise continued until exhaustion, defined as inability to maintain running speed despite repeated contact with the electric grid. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. Six days after training, mice were subjected to an endurance protocol, repeated once a week for 4 consecutive weeks, with belt running at accelerated speed. Mice were first acclimated with treadmill for 2 min, followed by a running session with belt speed initially set at 12 m/min. At 5 min after the initiation of exercise, the speed was increased by 1 m/min every 2 min and exercise continued until exhaustion. Mice were tested at 3 and 12 months of age.

Results & Conclusions

The WT mice were able to run for at least 30 min before reaching exhaustion, regardless of the age of the mice. This endurance performance remained almost constant for the four consecutive trials. Compared with WT mice, mdx mice showed a significantly reduced running time, which progressively decreased through the successive trials, with a cumulative endurance of about 15 min before reaching exhaustion. Jazz-negative mdx mice behaved similarly to mdx mice and, although they showed a cumulative endurance somewhat better than mdx mice, they also showed progressive worsening over the consecutive trials. It should be noted that the slightly higher value of cumulative endurance in Jazz-negative mdx compared with mdx mice was probably due to the different genetic background of these two mouse models. Indeed, mdx mice have a C57BL/6J background whereas mdx-Jazz mice have a mixed C57BL/6JxDBA background. Usually, C57BL/6J mice show significantly lower performance in treadmill exercises than do other strains, including DBA mice. The up-regulation of utrophin achieved by the expression of Jazz fully counteracted the deleterious effects of the dystrophic pathology, resulting in a significantly enhanced endurance performance in Jazz-positive mice, independent of the age of the mice.

Conclusions

Using in vitro testing methods, we showed that Jazz improves both muscle contractile force and sarcolemmal integrity in transgenic mdx mice. During a forced physical exercise on an accelerating treadmill, Jazz-positive mdx mice exhibited better exercise performance than Jazz-negative mdx and, surprisingly, they reached endurance levels similar to those recorded in healthy wild-type mice. Importantly, the enhanced performance of Jazz-positive mice was maintained throughout the life of the animals. This exclusive animal model establishes the notion that utrophin-based therapy for DMD can be efficiently developed using ZF ATF technology and candidates Jazz as a novel therapeutic molecule for DMD therapy.

Materials & Methods

We assessed the effects of Jazz on the force production of dystrophic muscles in vivo. Different groups of mice (WT, mdx and both Jazz-negative and Jazz-positive mdx mice), at 3 or 12 months of age, were subjected to forced physical exercise on an accelerating treadmill. The exercise was repeated once a week for four consecutive weeks and the running time was recorded in each session. Shock intensity was set at 0.4 mA. Inclination of treadmill was set at 0°. During the first session, mice were first familiarized to the treadmill apparatus for 2 min before starting the belt followed by a running with speed set at 6 m/min. Each mouse was immediately removed from treadmill after 3 electric shock. One day after habituation, mice were subjected to a training protocol with treadmill running at mixed speed. Mice were first acclimated to the treadmill apparatus for 2 min, followed by a running with speed initially set at 6 m/min. At 5, 15, 65, 75 min after the initiation of exercise, the speed was increased for 5 min by 0.6 m/min. At the end of this incremental procedure, the final speed was 18 m/min. Exercise continued until exhaustion, defined as inability to maintain running speed despite repeated contact with the electric grid. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. The time for removal of mice from the treadmill was 5 s on the shocker plate without attempting to reengage the mouse. Six days after training, mice were subjected to an endurance protocol, repeated once a week for 4 consecutive weeks, with belt running at accelerated speed. Mice were first acclimated with treadmill for 2 min, followed by a running session with belt speed initially set at 12 m/min. At 5 min after the initiation of exercise, the speed was increased by 1 m/min every 2 min and exercise continued until exhaustion. Mice were tested at 3 and 12 months of age.

Introduction

The absence of the cytoskeletal protein dystrophin results in Duchenne muscular dystrophy (DMD). The utrophin protein is the best candidate for dystrophin replacement in DMD patients. To obtain therapeutic levels of utrophin expression in dystrophic muscle, we developed an alternative strategy based on the use of artificial zinc finger transcription factors (ZF ATFs). The ZF ATF ‘Jazz’ was recently engineered and tested in vivo by generating a transgenic mouse specifically expressing Jazz at the muscular level. To validate the ZF ATF technology for DMD treatment we generated a second mouse model by crossing Jazz-transgenic mice with dystrophic-deficient mdx mice. Here, we show that the artificial Jazz protein restores sarcolemmal integrity and prevents the development of the dystrophic disease in mdx mice.

**EXPERIMENT SUMMARIZATION**

**Part #** | **Description** | **Model**
---|---|---
76-0309 | Mice 5-lanes Treadmill for mice, including shock source | LE8710M
76-0406 | SeDaCom V2.0 software for treadmill control and data transfer to a compute | SEDACOM V2.0
76-0609 | SeDaCom accessory – RS232/USB adapter | CONRS232USB
www.panlab.com
Presynaptic Control of Rapid Estrogen Fluctuations in the Songbird Auditory Forebrain with the Use of Microdialysis

Remage-Healey et al. (2010)
Presynaptic Calcium-Dependent Estrogen Flux, J. Neurosci., 31(27), 10034–10038 • 10037

Precautions

- Avoid contact with eyes, mouth, and skin. If contact occurs, rinse with water for at least 15 min. If irritation occurs, consult a physician.
- Keep away from children. Store at room temperature.
- Use in a well-ventilated area. Avoid inhalation.
- Wear protective clothing, gloves, and safety glasses when handling.

Materials & Methods

Surgery

A small incision was made in the outer and inner skull layers above NCM, and the dura mater was carefully opened. A CMA7 guide cannula (CMA Microdialysis) was implanted into NCM and cemented in place with cyanoacrylate and dental cement. The birds were returned to individual sound attenuation chambers for post surgical recovery (~5 d).

Microdialysis

Microdialysis probes CMA 7, cuprophane (CMA Microdialysis) were implanted at least 12 h before an experimental sampling session to allow behavioral adaptation and for acute implantation-induced phenomena to subside. The dummy probe was removed from the guide cannula and replaced by a CMA 7 microdialysis probe that had been prefilled with aCSF at a flow rate of 3 µL/min (CMA Microdialysis).

For high K+ experiment (n = 8), normal aCSF was perfused into NCM for two 30 min baseline sampling (pre1 and pre2), switched to high K+ aCSF solution for 30 min, followed by three 30 min washout periods of normal aCSF (wash 1, 2, and 3). For the Ca2+ experiment (n = 6), normal aCSF was perfused into NCM for two 30 min baseline sampling (pre1 and pre2), switched to a Ca2+-free aCSF solution for three 30 min periods (Ca2+-free 1, 2, and 3; a paradigm that induces steady removal of local extracellular Ca2+) (Westerink, 1995; Sasaki et al., 2006), followed by two 30 min washout periods of normal aCSF replacement (wash 1 and 2).

Results & Conclusions

Similar to findings with glutamate-evoked excitation (Remage-Healey et al., 2008), retrodialysis of aCSF containing high concentration K+ ions caused transient suppression of local E2 levels in NCM (Fig. 1A) (n = 7). The nonparametric repeated measures Friedman test revealed an overall effect of high K+ treatment on local E2 levels (X2 = 7.9; p = 0.068). Wilcoxon signed rank test loc tests determined that local E2 levels were significantly elevated by the third Ca2+-free treatment period (Z = -2.547; p < 0.01) relative to baseline (normal Ca2+ containing aCSF in pre2 sample). Once extracellular Ca2+ levels were restored (wash 1), local E2 levels returned to baseline (p = 0.05 for the transition from C Ca2+-free 3 to wash 1). Therefore, removal of extracellular Ca2+ levels within NCM was associated with a release of inhibition of local E2 levels, consistent with a Ca2+-dependent suppression of aromatase enzyme activity in NCM neurons.

Introduction

Anatomical localization of the estrogen synthesis enzyme (aromatase) within presynaptic terminals suggests that neuroestrogens can be synthesized directly at the neuronal synapse. A consequent prediction follows that synaptic estrogen production is controlled via classical electrochemical events in neurons. Here, we present evidence that acute fluctuations in local neuroestrogen levels in the forebrain of the zebra finch depend on calcium influx within presynaptic terminals. In vivo experiments using microdialysis linked to a sensitive estrogen ELISA showed that local forebrain neuroestrogens were both suppressed by potassium-evoked excitation and upregulated over 30 min periods of extracellular calcium depletion in a region enriched with presynaptic aromatase.

Materials & Methods

Surgery

A small incision was made in the outer and inner skull layers above NCM, and the dura mater was carefully opened. A CMA7 guide cannula (CMA Microdialysis) was implanted into NCM and cemented in place with cyanoacrylate and dental cement. The birds were returned to individual sound attenuation chambers for post surgical recovery (~5 d).

Microdialysis

Microdialysis probes CMA 7, cuprophane (CMA Microdialysis) were implanted at least 12 h before an experimental sampling session to allow behavioral adaptation and for acute implantation-induced phenomena to subside. The dummy probe was removed from the guide cannula and replaced by a CMA 7 microdialysis probe that had been prefilled with aCSF at a flow rate of 3 µL/min (CMA Microdialysis).

For high K+ experiment (n = 8), normal aCSF was perfused into NCM for two 30 min baseline sampling (pre1 and pre2), switched to high K+ aCSF solution for 30 min, followed by three 30 min washout periods of normal aCSF (wash 1, 2, and 3). For the Ca2+ experiment (n = 6), normal aCSF was perfused into NCM for two 30 min baseline sampling (pre1 and pre2), switched to a Ca2+-free aCSF solution for three 30 min periods (Ca2+-free 1, 2, and 3; a paradigm that induces steady removal of local extracellular Ca2+) (Westerink, 1995; Sasaki et al., 2006), followed by two 30 min washout periods of normal aCSF replacement (wash 1 and 2).

Results & Conclusions

Similar to findings with glutamate-evoked excitation (Remage-Healey et al., 2008), retrodialysis of aCSF containing high concentration K+ ions caused transient suppression of local E2 levels in NCM (Fig. 1A) (n = 7). The nonparametric repeated measures Friedman test revealed an overall effect of high K+ treatment on local E2 levels (X2 = 7.9; p = 0.068). Wilcoxon signed rank test loc tests determined that local E2 levels were significantly elevated by the third Ca2+-free treatment period (Z = -2.547; p < 0.01) relative to baseline (normal Ca2+ containing aCSF in pre2 sample). Once extracellular Ca2+ levels were restored (wash 1), local E2 levels returned to baseline (p = 0.05 for the transition from C Ca2+-free 3 to wash 1). Therefore, removal of extracellular Ca2+ levels within NCM was associated with a release of inhibition of local E2 levels, consistent with a Ca2+-dependent suppression of aromatase enzyme activity in NCM neurons.
Introduction

The expression of the Dlx homeobox genes is closely associated with neurons that express γ-aminobutyric acid (GABA) in the embryonic rostral forebrain. To test whether the Dlx genes are sufficient to induce some aspects of the phenotype of GABAergic neurons, electroporation method was adapted to ectopically express Dlx proteins in slice cultures of the mouse embryonic cerebral cortex. This approach showed that ectopic expression of Dlx2 and Dlx5 induced the expression of glutamic acid decarboxylases (GADs), the enzymes that synthesize GABA. This method was also used to show cross-regulation between different Dlx family members. It was found that Dlx1 can induce Dlx5 expression, and that Dlx1, Dlx2 and Dlx5 can induce expression from a Dlx5/6-lacZ enhancer/reporter construct.

Materials & Methods

A tissue vibratome slice (in 4% low melting point agarose in 1x Krebs), with its supporting membrane (Nucleopore Track-Etch membrane, Whatman), was placed onto a 1% agarose block (in 1x Krebs buffer) within a setup of two horizontally oriented platinum electrodes. A small amount of plasmid solution was applied to the lower end of the agarose column, and then the electrode was lowered to let the solution contact the tissue. Using the ECM 830 generator, 2 pulses at 125 volts for 5 milliseconds each, was applied to the explant tissue slices.

Results & Conclusions

Counting of GFP- and Dlx-positive cells showed that >95% of electroporated cells expressed both plasmids. In the most effective Dlx2 electroporations, more than 85% of the GFP positive cells expressed GAD65. Dlx5 was less efficient at inducing GAD65, with the most effective electroporations inducing GAD65 in ~50% of the GFP positive cells. Slice culture electroporation is spatially more precise and it can be readily applied to diverse species. Given the large number of mouse mutants now available, this opens the possibility of rescuing mutant phenotypes. Furthermore, many developmental and physiological processes are largely unperturbed in slice cultures (e.g., neuronal migrations)(Anderson et al., 1997b; Anderson et al., 2001); thus electroporation that does not restrict the size of the transfected plasmid (unlike most viral vectors) will be an effective method with which to study rapidly the effects of genes on specific processes.
Use of Syringe Pumps for Injection into Brain Tumors & Tumor Sites


Controlling brain tumor growth by intraventricular administration of an AAV vector encoding IFN-β: ICV delivery of AAV to control brain tumors,

*Brain Research* 16, 664-671

**Introduction**

Glioblastoma multiforme (GBM) is the most aggressive type of all primary brain tumors, with an overall median survival < 1 year after diagnosis. Despite introduction of multimodal treatment approaches, the prognosis has not improved significantly over the last 50 years. In this study we investigated the effect of intracerebroventricular injection of an AAV vector encoding human interferon-beta (hIFN-β) on glioblastoma growth.

Recently, we found that peri-tumoral parenchymal transduction with an AAV vector encoding hIFN-β was exceptionally efficient in eradicating GBM brain tumors. Here we show that pretreatment of mice via intracerebroventricular (ICV) infusion of an AAV vector encoding hIFN-β (AAV-IFN-β) completely prevents tumor growth in an orthotopic model of GBM. We also show that pretreatment of mice via intracerebroventricular (ICV) injection of an AAV vector encoding human interferon-beta (hIFN-β) on glioblastoma growth.

**Materials & Methods**

Intracranial injections were performed in male BALB/c nu/nu mice age 6–8 weeks obtained from a colony maintained at the Massachusetts General Hospital. Mice were anesthetized by intraperitoneal injection of ketamine (125 mg/kg) and xylazine (12.5 mg/kg) in 0.9% saline and placed in a rodent stereotaxic frame (Stoelting, Wood Dale, IL). Tumor cells were implanted in the left or right midstriatum using the following coordinates from bregma in mm: (AP +0.5, ML +2.0, DV -2.5). AAV vectors were infused into the left lateral ventricle (coordinates from bregma in mm: AP -0.4, ML +1.0, DV -1.7) using a Harvard 22 syringe pump (Harvard Apparatus, MA) to drive a gas-tight Hamilton Syringe (Hamilton Co., Rena, NV) attached to a 33-gauge steel needle (Hamilton Co., Rena, NV) via PEEK tubing (Alltech, Deerfield, IL) and Luer adapters (Amersham Biosciences, Piscataway, NJ). Briefly, 5×10^10 genome copies (gc) or 5×10^11 gc were infused at a rate of 4 μl/min after which the needle was left in place for 2 min to prevent backflow before withdrawal. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Massachusetts General Hospital and follows guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For the survival analysis experiments, animal death was defined as euthanasia after the development of symptoms associated with tumor burden in accordance with the guidelines of the MGH animal facility.

**Results & Conclusions**

Pre-treatment of mice by intraventricular injection of AAV-hIFN-β prevents U87 tumor growth. Proof-of-principle experiments showing prevention of glioma growth after intraventricular AAV-hIFN-β injection.

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**EXPERIMENT SUMMARIZATION**

**Materials & Methods**

Intracranial injections were performed in male BALB/c nu/nu mice age 6–8 weeks obtained from a colony maintained at the Massachusetts General Hospital. Mice were anesthetized by intraperitoneal injection of ketamine (125 mg/kg) and xylazine (12.5 mg/kg) in 0.9% saline and placed in a rodent stereotaxic frame (Stoelting, Wood Dale, IL). Tumor cells were implanted in the left or right midstriatum using the following coordinates from bregma in mm: (AP +0.5, ML +2.0, DV -2.5). AAV vectors were infused into the left lateral ventricle (coordinates from bregma in mm: AP -0.4, ML +1.0, DV -1.7) using a Harvard 22 syringe pump (Harvard Apparatus, MA) to drive a gas-tight Hamilton Syringe (Hamilton Co., Rena, NV) attached to a 33-gauge steel needle (Hamilton Co., Rena, NV) via PEEK tubing (Alltech, Deerfield, IL) and Luer adapters (Amersham Biosciences, Piscataway, NJ). Briefly, 5×10^10 genome copies (gc) or 5×10^11 gc were infused at a rate of 4 μl/min after which the needle was left in place for 2 min to prevent backflow before withdrawal. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Massachusetts General Hospital and follows guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. For the survival analysis experiments, animal death was defined as euthanasia after the development of symptoms associated with tumor burden in accordance with the guidelines of the MGH animal facility.

**Products from Harvard Apparatus used in this study:**

- **Part #**
  - 55-2222 Pump 22 Syringe Pump

**Products**

- **Part #**
  - 55-2222 Pump 22 Syringe Pump

**Website**

[www.harvardapparatus.com](http://www.harvardapparatus.com)
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