VINBLASTINE ACUTELY SENSITIZES HEMATOPOIETIC CANCERS TO BCL-2 TARGETING DRUGS

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Chronic lymphocytic leukemia (CLL) characteristically maintains high levels of the anti-apoptotic protein Bcl-2, thus protecting them from apoptotic death and leading to an accumulation of mature resting B cells. Many other hematopoietic malignancies including acute promyelocytic leukemia (APL) also depend upon Bcl-2 family proteins for survival, suggesting that Bcl-2 proteins are excellent targets for anti-cancer therapies. We have previously shown that some leukemia and lymphoma cell lines undergo rapid apoptosis (≤6 h) when incubated with the microtubule disrupting agent vinblastine. Several other cell lines undergo this rapid apoptosis when vinblastine is combined with various approaches that target the anti-apoptotic proteins Bcl-2 or Mcl-1. These approaches include incubation with ABT-737 that inhibits Bcl-2 and Bcl-X, and SCH727965 (dinaciclib) that inhibits CDK7/9, thereby inhibiting translation, which results in rapid loss of Mcl-1. In this study, we have assessed the ability of these agents alone or in combination to induce apoptosis in freshly isolated CLL and APL cells. Incubation with vinblastine alone induced variable apoptosis within 6 h (5-60%). ABT-737 induced rapid and almost complete apoptosis in CLL and in 50% of APL cells ex vivo. However, when combined with vinblastine, a 10-fold lower concentration of ABT-737 was required to induce complete apoptosis. Dinaciclib alone also induced variable levels of apoptosis (5-90%), but again the combination with vinblastine induced extensive apoptosis at lower concentrations of both drugs. Normal peripheral blood mononuclear cells were resistant to single agent or combination treatments. Sensitivity to ABT-737, dinaciclib or vinblastine was independent of CD38 and ZAP70 status and previous clinical treatment (untreated or relapsed patients). These results suggest that combinations of vinblastine and ABT-737 or dinaciclib are more effective than single agent therapy. Furthermore, these combinations may overcome resistance to individual drug therapies in patients with CLL or APL.
FEAR CONDITIONING IS DISRUPTED BY DAMAGE TO THE POSTSUBICULUM

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The hippocampus plays a central role in spatial and contextual learning and memory, however relatively little is known about the specific contributions of parahippocampal structures that interface with the hippocampus. The postsubiculum (PoSub) is reciprocally connected with a number of hippocampal, parahippocampal and subcortical structures that are involved in spatial learning and memory. In addition, behavioral data suggest that PoSub is needed for optimal performance during tests of spatial memory. Together, these data suggest that PoSub plays a prominent role in spatial navigation. Currently it is unknown whether the PoSub is needed for other forms of learning and memory that also require the formation of associations among multiple environmental stimuli. To address this gap in the literature we investigated the role of PoSub in Pavlovian fear conditioning. In Experiment 1 male rats received either lesions of PoSub or Sham surgery prior to training in a classical fear conditioning procedure. On the training day a tone was paired with foot shock three times. Conditioned fear to the training context was evaluated 24 hr later by placing rats back into the conditioning chamber without presenting any tones or shocks. Auditory fear was assessed on the third day by presenting the auditory stimulus in a novel environment (no shock). PoSub-lesioned rats exhibited impaired acquisition of the conditioned fear response as well as impaired expression of contextual and auditory fear conditioning. In Experiment 2, PoSub lesions were made 1 day after training to specifically assess the role of PoSub in fear memory. No deficits in the expression of contextual fear were observed, but freezing to the tone was significantly reduced in PoSub-lesioned rats compared to shams. Together, these results indicate that PoSub is necessary for normal acquisition of conditioned fear, and that PoSub contributes to the expression of auditory but not contextual fear memory.
Methicillin Resistant *Staphylococcus aureus* (MRSA) represent a bacterial species endemic to hospitals and are capable of causing a wide spectrum of diseases. The major concern with MRSA is the rapid evolution of multiple drug resistance, which restricts the use of currently available treatment options. MRSA infections in hospitals have virtually doubled nationwide over the last decade, with deaths increasing from 11,000 to more than 17,000, which is higher than those dying of HIV/AIDS. Moreover, the incidence of community-acquired MRSA (CA-MRSA) infections has increased substantially over the last five years in healthy individuals without any known risk factors. With antibiotic resistances growing so quickly, it is extremely important that we stay ahead of this disturbing trend by identifying new antimicrobial targets. To identify novel underlying resistance mechanisms that the bacteria invoke when in contact with the drug, Imipenem, we have used whole genome sequencing and identified mutations in *apt* (adenine phosphoribosyl transferase) and *prs* (ribose phosphate pyrophosphokinase), both genes involved in the purine salvage pathway. We propose that a mutation in these genes makes the cells hypermutable and easily adaptable to antibiotic stress. Elucidation of the drug resistance mechanism will aid in selecting better targets for antibiotics. In a related research endeavor, we have shown that resistance to drugs like β-lactams in CA-MRSA is *php4* dependent, where *php4* is a penicillin binding protein involved in peptidoglycan synthesis. By exploiting this phenotype we have successfully scanned a small percentage of the compounds in small molecule library at the ICCB Longwood, to identify compounds that disrupt the function of *php4* and thus have therapeutic potential.
Computer simulations of plasma turbulence and particle and heat transport in a dipole magnetic field geometry created by a ring current are presented [Kobayashi et al, Physical Review Letters, 2010]. This study is relevant to the MIT/Columbia University Levitated Dipole Experiment (LDX) [Kesner et al, Plasma Phys. Reports, 1997], a fusion experiment designed to explore hot plasma confinement in a dipolar magnetic field. The work also has potential applications to planetary magnetospheres. In addition to magnetohydrodynamic (MHD) ideal interchange and ballooning modes, a non-MHD mode known as the entropy mode is present in this system. The entropy mode has a scale length smaller than ideal modes ($k_\perp \rho_i \sim 1$) but comparable growth rates. Considering parameter regimes that are ideally stable, we explore the physics of turbulent transport generated by entropy modes, finding enormous variation in the nonlinear dynamics as a function of the density and temperature gradients. In particular, we report here the existence a new particle pinch regime, in which the particles are transported up the density gradient. We show that this discovery is consistent with gyrokinetic and two-fluid quasi-linear theory. The presence of a particle pinch appears to be consistent with recent observations in LDX [Boxer et al, Nature Physics, 2010].
THE MRE11 NUCLEASE IS CRITICAL FOR THE SENSITIVITY OF CELLS TO CHK1 INHIBITION

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The Chk1 kinase is required for the arrest of cell cycle progression when DNA is damaged, and for stabilizing stalled replication forks. As a consequence, many Chk1 inhibitors have been developed and tested for their potential to enhance DNA damage-induced tumor cell killing. However, inhibition of Chk1 alone, without any additional exogenous agent, can be cytotoxic. Understanding the underlying mechanisms of this sensitivity is critical for defining which patients might respond best to therapy with Chk1 inhibitors. We have investigated the mechanism of sensitivity in U2OS osteosarcoma cells. Upon incubation with the Chk1 inhibitor MK-8776, single-stranded DNA regions (ssDNA) and double-strand breaks (DSB) begin to appear within 6 h. These DSB have been attributed to the structure-specific DNA endonuclease, Mus81. The Mre11/Rad50/Nbs1 (MRN) complex is known to be responsible for the resection of DSB to ssDNA. However, we show that inhibition of the Mre11 nuclease activity leads, not only to a decrease in the amount of ssDNA following Chk1 inhibition, but also inhibits the formation of DSB, suggesting that DSB are a consequence of ssDNA formation. These findings were corroborated by the discovery that Mre11-deficient ATLD1 cells are highly resistant to MK-8776 and form neither ssDNA nor DSB following treatment, but once complimented with exogenous Mre11, the cells accumulate both ssDNA and DSB when incubated with MK-8776. Our findings suggest that Mre11 provides the link between aberrant activation of Cdc25A/Cdk2 and Mus81. The results highlight a novel role for Mre11 in the production of DSB and may help define which tumors are more sensitive to MK-8776 alone or in combination with DNA damaging agents.
TEMPORAL DYNAMICS OF SPATIAL WORKING MEMORY: EFFECTS OF EVENT-RELATED DEEP BRAIN STIMULATION OF VENTRAL MIDLINE THALAMUS IN RATS

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Permanent or temporary damage to reuniens nuclei (Re) in the ventral midline thalamus produces impairments in spatial working memory. To determine whether there is a specific phase in memory processing for which proper functioning of Re is necessary, we examined the effects of deep brain stimulation in Re at different phases of a delayed non-match to position (DNMTP) task. Stimulation was applied during one of four phases of DNMTP corresponding to different memory processes: initiation (planning), sample (encoding), delay (storage), and choice (retrieval). Low (0.01mA) and high (0.03-0.4mA) current levels were used for stimulation where high current values were individually set below spontaneous behavior-inducing levels. DNMTP was tested for two delays (3 and 15 sec) that were randomly selected on a trial-by-trial basis. Results indicate a significant overall effect of stimulation level on working memory (F_{2, 12} = 103.57, p<0.0001). Further analyses support significant working memory impairments for high current stimulation compared to low or no current stimulation sessions (p<0.0001). Average performance on no-stimulation trials was 82.2% correct compared to 56.3% correct on high current stimulation trials. Deficits were significant during specific phases of the task, namely the delay (p=0.0028) and choice phases (p=0.0005) for the 3-second delay. Finally, we compared the effects of stimulation on performance in two different memory tasks, spatial reference memory (SRM) and DNMTP, with identical choice responses. There was no effect of high current stimulation on performance in the SRM task; average performance was 86.6% correct for no-stimulation and 87.5% correct for high stimulation. Our data suggest that performance on the working memory task (DNMTP) is influenced by stimulation while performance on the reference memory task (SRM) is not. Taken together, these results suggest that Re specifically affects working memory processes involved in representing information during brief memory delays and in executing memory-guided responses.
THE ROLE OF TYPE-B RESPONSE REGULATORS IN THE MERISTEMS OF ARABIDOPSIS AND RICE

Ian H. Street, Department of Biological Sciences (PI: G. Eric Schaller)

Objective I: Test the hypothesis that type-B RRs localize within the apical meristems
I am analyzing GUS staining patterns in native promoter type-B RR:GUS translational fusions. I have generated one entry clone for a rice type-B RR, OsRR22 ready to be recombined into a destination vector to create a GUS fusion and subsequent transformation into rice.

Objective II: Test the hypothesis that type-B RRs have a functional role in apical meristems
I have generated GATEWAY destination vectors containing meristem specific promoters (p) pWUS, pCLV1, pCLV3, pSTM, pCUC2, pWOX5 and pARR12 driving a GATEWAY cassette. I have recombined in a genomic copy of ARR12 as well as an ARR1,12 tandem RNAi clone. pWOX5 driving a genomic copy of ARR10 has also been made. I have selected T1 plants in an arr1,10,12 triple mutant with the promoter:ARR12 fusions as well as promoter:arr1,12 RNAi clones in the wild type and ARR10 knockout backgrounds. Initial analysis suggests that the control construct pARR12:ARR12 construct is able to rescue the phenotype of the arr1,10,12 triple mutant. pWOX5:ARR10 T1 plants are able to rescue the aborted primary root of the arr1,10,12 triple mutant, suggesting a role for cytokinin in maintaining the root apical meristem.

Objective III: Test the hypothesis that type B RRs and WUSCHEL and the rice WUSCHEL homolog OsWOX11, directly co-regulate type-A RRs
I have initial results from a protoplast assay using WUS and ARR12 expressed together and alone targeting the promoter of ARR6 fused to luciferase (pARR6:LUC). In this assay, WUS does not appear to repress the pARR6:LUC activity alone or impair the ability of ARR12 to induce the luciferase reporter. Yeast-2-hybrid clones are being generated to test the hypothesis that type-B RRs and WUS physically interact.
THE PIONEER FACTOR PBX1 GUIDES A DISTINCT ERA SIGNALING IN BREAST CANCER

Luca Magnani, Department of Genetics (PI: Mathieu Lupien)

Altered transcriptional programs are a hallmark of diseases, yet how these are established is still ill-defined. PBX1 is a TALE homeodomain protein involved in the development of different types of cancers. The estrogen receptor alpha (ERα) is central to the development of two-thirds of all breast cancers. Here we demonstrate that PBX1 acts as a pioneer factor and is essential for the ERα-mediated transcriptional response driving aggressive tumors in breast cancer. Indeed, PBX1 expression correlates with ERα in primary breast tumors, and breast cancer cells depleted of PBX1 no longer proliferate following estrogen stimulation. Profiling PBX1 recruitment and chromatin accessibility across the genome of breast cancer cells through ChIP-seq and FAIRE-seq reveals that PBX1 is loaded and promotes chromatin openness at specific genomic locations through its capacity to read specific epigenetic signatures. Accordingly, PBX1 guides ERα recruitment to a specific subset of sites. Expression profiling studies demonstrate that PBX1 controls over 70% of the estrogen response. More importantly, the PBX1-dependent transcriptional program is associated with poor-outcome in breast cancer patients. Correspondingly, PBX1 expression alone can discriminate a priori the outcome in ERα-positive breast cancer patients. These features are markedly different from the previously characterized ERα-associated pioneer factor FoxA1. Indeed, PBX1 is the only pioneer factor identified to date that discriminates outcome such as metastasis in ERα-positive breast cancer patients. Together our results reveal that PBX1 is a novel pioneer factor defining aggressive ERα-positive breast tumors, as it guides ERα genomic activity to unique genomic regions promoting a transcriptional program favorable to breast cancer progression.
COMBINED FLUORESCENCE AND REFLECTANCE SPECTROSCOPY FOR 
IN VIVO QUANTIFICATION OF CANCER BIOMARKERS IN LOW- AND 
HIGH-GRADE GLIOMA SURGERY

Pablo A. Valdes, Department of Surgery (PI: Keith Paulsen and David W. Roberts)

Gliomas represent a heterogeneous group of brain tumors, with extent of resection shown as a significant factor influencing post-surgical recurrence and prognosis. Recently, use of 5-aminolevulinic acid (ALA)-induced protoporphyrin IX (PpIX) fluorescence-guided resection has shown promise for improving the degree of resection in high-grade gliomas. We have shown that absolute quantification of PpIX biomarker in tissue significantly improves tumor detection across a range of tumor histologies. Nevertheless, despite improved detection for tumor tissue with this biomarker, and as a result of the inter- and intra-tumor heterogeneity of gliomas and multifaceted nature of neoplastic processes, individual biomarkers are not sufficient for achieving optimal tumor tissue diagnosis. Here, we hypothesized that use of multiple factors (i.e., biomarkers) is necessary to maximize the diagnostic and predictive power of optical technologies for tumor tissue delineation. We used fluorescence and reflectance spectral signatures for in vivo quantification of multiple biomarkers during glioma surgery, with fluorescence contrast provided by exogenously-induced PpIX following administration of ALA. We performed light-transport modeling to quantify multiple biomarkers indicative of tumor biological processes intraoperatively, including the local concentration of PpIX and associated photoproducts, total hemoglobin concentration, oxygen saturation and optical scattering parameters. We developed a diagnostic algorithm for intraoperative tissue delineation that accounts for the combined tumor-specific predictive capabilities of these quantitative biomarkers. Tumor tissue delineation achieved accuracies of up to 94% (specificity = 94%, sensitivity = 94%) across a range of glioma histologies beyond current state-of-the-art optical approaches, including state-of-the-art fluorescence image guidance. These results suggest the ability of a quantitative multiple biomarker approach to detect both tumor bulk and infiltrating glioma tissues, especially in low-grade gliomas where the impact on patient prognosis and survival could be substantial. This multiple biomarker strategy opens the door to optical methods for surgical guidance that use in vivo quantification of well-established neoplastic processes.
THE CIRCADIAN PERIOD GENES MODULATE ALCOHOL CONSUMPTION AND THE EFFECTS OF CLOZAPINE ON ALCOHOL DRINKING IN A SEX-DEPENDENT MANNER

Joshua Gamsby, Department of Genetics (PI: Jay Dunlap)

The circadian clock governs the timing of many behavioral processes, such as the sleep/wake cycle. This clock is composed of a network of gene products that form a transcriptional/translational negative feedback loop, which includes the Per family of transcriptional repressors. Mounting evidence suggests a link between genetic perturbations of the core clock components and behavioral disorders, such as depression, alcoholism, and schizophrenia. For example, mice lacking the Per2 gene have previously been shown to have an increase in alcohol preference as compared to their wild type littermates. Furthermore, treatment with acamprosate, a glutamate system antagonist that has been used to decrease the symptoms of alcohol withdrawal, has been shown to diminish alcohol preference in these animals. As with acamprosate, the antipsychotic clozapine (CLOZ) reduces alcohol intake in patients and in animal models. This study examined the effects of CLOZ on free access alcohol drinking in alcohol-preferring C57BL/6J mice, as well as in mice deficient in Per1 (mPer1brdm1), Per2 (mPer2brdm1), and a double mutant of both mutant alleles.
DIFFERENTIAL PATTERNS OF DEACTIVATION WITHIN THE DEFAULT MODE NETWORK DURING PERFORMANCE OF A VERBAL WORKING MEMORY TASK IN PATIENTS WITH MULTIPLE SCLEROSIS

Evelyn J. Reilly, Departments of Psychiatry and Neurology (PI: Heather A. Wishart)

Task induced deactivation (TID) refers to decreased blood oxygenated level dependent signal during task performance as compared to rest. TID is commonly evidenced during attention demanding tasks within the default mode network (DMN). Increased short-term memory load significantly increases TID in healthy controls (HC: McKiernan et al., 2003) and greater magnitudes of TID in HC predict better memory performance (Wig et al., 2008). This phenomenon suggests that successful performance of cognitively demanding tasks requires suspension of spontaneous processes that occur during resting state. Studies of MS have evidenced decreased and more dispersed TID in patients relative to HC (Morgen et al., 2007; Genova et al., 2009) and dysfunction in several DMN regions during resting state fMRI (Rocca et al., 2010; Bonavita et al., 2010). TID during performance of a verbal working memory (VWM) task has not yet been explored in intact versus impaired patients with MS. We hypothesized that HC would show greater TID of the DMN than MS patients with “High-P” cognition, and “High-P” patients would show greater TID than MS patients with “Low-P” cognition. Participants included 15 HC and 33 patients with relapsing-remitting MS, subgrouped into “High-P” (n=14) and “Low-P” (n=19) groups based on 3T in-scanner performance during an auditory 3-back task in addition to out-of-scanner neuropsychological test performance. Following detrending, images were realigned, normalized, and smoothed using SPM5. Age, gender, and education were used as covariates in the SPM analyses. Task greater than rest conditions showed expected activation of canonical VWM circuitry and all groups showed TID within the DMN. ANCOVA using rest>task contrasts in the DMN region of interest (voxel-level critical p < 0.001, k=3) revealed widespread increases of TID within the DMN in patients with “Low-P” VWM as compared to the HC and, to a lesser extent, the “High-P” group at each level of task difficulty. Furthermore, the “Low-P” group showed larger regions and higher intensity of TID with increasing task difficulty, as compared to both groups, despite a decline in performance. The HC and MS “High-P” group showed only small isolated regions of greater TID than the “Low-P” group at each task level. These findings indicate that, contrary to expectations, impaired VWM performance in MS is associated with increased TID of the DMN. These findings will be discussed in the context of the emerging literature on TID in MS.

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HYPOXIC VENTILATORY RESPONSE IN RAT PUPS EXPOSED TO DIETARY TRYPTOPHAN DEFICIENCY

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Many Sudden Infant Death Syndrome (SIDS) cases are associated with abnormalities in the medullary serotonergic system. We hypothesize that these abnormalities impair infants from adequately responding to stresses, such as hypoxia and hypercapnia, which may be a consequence of rebreathing exhaled gases when in the prone position. To examine the role of a moderate depletion of serotonin (5-HT) on ventilatory responses to hypoxia, we studied pups born from Sprague-Dawley dams fed a diet partially deficient in tryptophan (~45%). Using head-out plethysmography, no significant differences were detected in baseline tidal volume (VT), breathing frequency (FR), or ventilation (VE) at any age (postnatal days (P) 5, 8, 12). We measured the average ventilatory response to hypoxia by exposing pups to 10 alternating episodes of hypoxia (10% O2, 1 min) and room air (5 min). At P5, tryptophan deficient diet pups exhibited an enhanced VT and VE response to hypoxia when compared to control (P=0.011; P=0.007). At P8, VT responses were similar between both groups, however the FR and VE responses in diet pups were significantly higher than control (P=0.022; P=0.041). No differences were observed between the two groups at P12. We also observed post-hypoxic long-term ventilatory depression in controls at P5 and ventilatory facilitation at P8 and P12, with no similar effect in tryptophan deficient pups. Results suggest that alterations in dietary tryptophan leads to abnormal ventilatory responses to hypoxia during a sensitive period in development which may be relevant to our understanding of SIDS, where brainstem 5-HT abnormalities could lead to disruption of homeostatic responses.
SYNERGISTIC EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL AND CANNABIDIOL IN REDUCING ALCOHOL DRINKING AND WEIGHT GAIN IN THE SYRIAN GOLDEN HAMSTER

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**Introduction:** Clinical and preclinical studies suggest that the psychoactive cannabinoid delta-9-tetrahydrocannabinol (THC), a cannabinoid-1 (CB1) receptor partial agonist, worsens or even induces psychosis in patients with schizophrenia. However, the cannabinoid cannabidiol (CBD) may actually reverse these effects and act as an antipsychotic agent. Cannabinoids have also been shown to mediate alcohol abuse, and we hypothesized that CBD would reduce alcohol intake as well as improve the symptoms of schizophrenia, making it a putative treatment for co-occurring schizophrenia and alcohol use disorder. Thus, the current studies examined whether chronic exposure to THC or CBD could alter alcohol intake. We tested these drugs in the Syrian golden hamster, which shows similar alcohol drinking patterns to patients with schizophrenia.

**Methods:** Hamsters were given free access to alcohol (15% v/v) and water; once alcohol consumption reached a steady baseline, hamsters were randomized into seven groups and treated with either: Vehicle (VEH), CBD (5-40 mg/kg) or THC (1-20 mg/kg) for one week, then the doses were doubled for each experimental group for an additional week. Finally, we added 40 mg/kg CBD to each THC-treated group for a third week. **Results:** THC dose-dependently decreased alcohol intake compared to VEH controls, producing a robust, persistent inhibition of alcohol consumption at the two higher doses tested. CBD failed to decrease alcohol intake, but potentiated the effects of low-dose THC in reducing alcohol intake. Interestingly, despite steady food intake, THC also blocked normal weight gain, and this effect was potentiated by concurrent CBD treatment. **Conclusions:** Although CB1 receptor agonists have been shown to increase alcohol craving and intake, the CB1 receptor partial agonist THC actually decreased alcohol intake, and this effect was potentiated by CBD. A combination therapy with a high ratio of CBD:THC may prove effective in treating co-occurring schizophrenia and alcohol use disorder.
Rats familiar with a 76 cm square apparatus were introduced into a novel 76 cm diam. cylinder. We recorded hippocampal place cell sets during a 10 min session in the square and subsequently in two 10 min sessions in the cylinder. We compared the discharge of the place cells in the two environments and in general found complete (global) remapping rather than rate remapping, even though the two arenas were located within the same sound-proof room. We also noted a distinct form of warm-up for a substantial fraction of cells in the novel cylinder; these cells fired very little at the beginning of the first cylinder session but became more active with time. There was much less evidence of a similar effect in the second cylinder session. Thus, the generation of a new spatial representation in the hippocampus is almost immediate but its resolution improves with time and is fully developed after ~5 - 7 min.
Anabolic androgenic steroids (AAS) are synthetic derivatives of testosterone illicitly taken for image and athletic improvement. A noted side effect of AAS use in humans is elevated levels of anxiety. We have shown that long-term (4 weeks), but not acute, exposure of female mice during adolescence to a high-dose mixture of three commonly abused AAS (nandrolone decanoate, testosterone cypionate, and methandrostenolone) augments anxiety-like behaviors as measured by the acoustic startle response and on the elevated plus maze. The anxiogenic effect of chronic AAS exposure occurs via a mechanism whereby AAS promote increased expression of corticotropin releasing factor (CRF) in the extended amygdala and enhanced CRF-dependent GABA-A receptor-mediated inhibition of neurons in the dorsal lateral bed nucleus of the stria terminalis (dlBnST). Whole-cell patch clamp recordings of brain slices containing the dlBnST indicated that AAS treatment resulted in an increase in the frequency, but not the amplitude or kinetics, of spontaneous inhibitory postsynaptic currents (sIPSCs) in dlBnST neurons. No effect of AAS treatment was observed on the amplitude, kinetics or frequency of miniature IPSCs recorded in the presence of 1µM tetrodotoxin, suggesting that the observed increase in the frequency of sIPSC occurred via a pre-synaptic, activity-dependent mechanism. The acute effects of AAS and CRF were also assessed in recordings made from neurons in the dlBnST of naïve adult female mice. Acute application of CRF increased the frequency, but did not change the amplitude or kinetics, of sIPSCs in dlBnST neurons. Conversely, acute application of the CRF-R1 antagonist significantly decreased sIPSC frequency in slices from AAS-treated animals to the level observed in oil-injected mice. Moreover, there was no difference in action potential frequency in dlBnST neurons of AAS-treated and oil-injected mice in the presence of GABA-A receptor antagonist, picrotoxin. These data are consistent with the proposed mechanism by which AAS promote enhanced presynaptic release of GABA via a CRF-mediated mechanism. As has been reported previously for other populations of forebrain neurons (for review, Henderson, 2007), acute application of 1 µM AAS (a concentration that reflects estimated CSF levels in AAS users) increased the amplitude of sIPSCs via allosteric modulation of GABA-A receptors in dlBnST neurons. Thus, in intact animals exposed to chronic steroids, AAS are likely to enhance GABAergic inhibition in the dlBnST via both pre- and postsynaptic mechanisms and this increased inhibition promotes an anxiety-like state in the female mice. Supported by DA14137, and T32DK07508.
Tightly controlled regulation of bacterial virulence genes is required for the successful survival and establishment of non-obligate bacterial pathogens in the diverse niches they colonize. *Vibrio cholerae* is the etiological agent of the severe diarrheal disease cholera, and is a natural inhabitant of estuarine and brackish waters. Two chief regulators (ToxR and TcpP) control the expression of ToxT, the master regulator of the *V. cholerae* virulence cascade. Both ToxR and TcpP are localized in the inner-membrane and their cytoplasmic domains interact with the *toxT* promoter region to activate its transcription. ToxT induces, among others, the transcription of the genes that encode the main pathogenicity factors of *V. cholerae* O1: the cholera toxin (CT), which is the cause of the massive diarrhea, and the toxin co-regulated pilus (TCP), an essential intestinal colonization factor. TcpP is always found associated with TcpH, which protects TcpP from protease degradation, and ToxR is associated with ToxS, whose function remains irresolute. Recently, it was shown that TcpP undergoes regulated intramembrane proteolysis (RIP) when *V. cholerae* is switched from inducing to non-inducing conditions. This process is dependent on the presence of YaeL, a protease localized to the inner-membrane. In this study we show that periplasmic truncations of ToxR induce its proteolysis in a YaeL-dependent manner. Interestingly, from the numerous conditions we tested, we could not find any that naturally induces proteolysis of ToxR. Nonetheless, we determined that a mutation in *toxS* induces partial proteolysis of both ToxR and TcpP in a YaeL-dependent manner, behaving oppositely from its counterpart, TcpH. This ToxS point mutant, ToxSL33S, is unable to autoagglutinate and has severely reduced production of the major pilin subunit of TCP, TcpA, and TcpF, a secreted colonization factor of *V. cholerae*. Overall, in this study we assign a possible function to ToxS and we uncover an extra layer in the complexity of this elaborate regulatory network by showing the first example of interaction between the ToxR/S and TcpP/H virulence regulatory complexes in *V. cholerae*. 
SGK1 INCREASES PLASMA MEMBRANE CFTR BY PHOSPHORYLATING SHANK2E IN HUMAN AIRWAY EPITHELIAL CELLS

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Cystic fibrosis (CF) is caused by mutations in CFTR, the cystic fibrosis transmembrane conductance regulator. The most common mutation, CFTR-ΔF508, leads to severely reduced abundance of CFTR in the plasma membrane, thus many therapeutic approaches aim to increase the amount of membrane CFTR. The synthetic corticosteroid dexamethasone (Dex) up-regulates serum- and glucocorticoid-induced protein kinase (SGK1) mRNA and protein and has been previously reported to augment the amount of CFTR in the plasma membrane of Xenopus oocytes (Sato et al., 2007) and CFPAC-1 pancreatic cells (Caohuy et al., 2009). However, the effect of Dex and SGK1 on plasma membrane CFTR levels in airway cells has not been reported. The goal of this study was to test the hypothesis that Dex, via up-regulation of SGK1, increases plasma membrane wt-CFTR in a human bronchial epithelial cell line (CFBE-wt) and to elucidate the underlying mechanism. Dex increased plasma membrane wt-CFTR by 139% (P<0.05), and increased SGK1 mRNA and protein by 171% and 708%, respectively (P<0.05). Because CFTR has four partial SGK1 consensus phosphorylation sites, we analyzed whether SGK1 increases membrane CFTR by direct phosphorylation. However, SGK1 did not directly phosphorylate CFTR and we therefore investigated whether an intermediate protein up-regulates membrane CFTR upon phosphorylation by SGK1. We chose to study Shank2, a PDZ domain containing scaffold protein, because recent studies have shown that Shank2 binds to CFTR (Lee et al. 2007) and that overexpression of Shank2 increases CFTR in the plasma membrane of NIH 3T3 cells (Kim et al. 2004). We found that the epithelial isoform Shank2E, which contains two SGK1 consensus phosphorylation sites, is endogenously expressed in CFBE-wt cells. siRNA against Shank2 reduced the Dex-mediated increase in plasma membrane CFTR by 55% (P<0.05). In addition, overexpression of a Shank2E mutant, in which the SGK1 phosphorylation sites were deleted, had a dominant-negative effect and reduced the Dex-induced increase in plasma membrane CFTR by 56% (P<0.05). Taken together, these findings suggest that Dex induces up-regulation of SGK1, which subsequently phosphorylates Shank2E, leading to increased surface expression of wt-CFTR, perhaps by stabilizing it at the membrane.