

9th Annual Robert J. Huskey Graduate Research Exhibition

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Seeing the Trees but Distorting the Forest: Global versus Local Memory in Social Anxiety

Meghan Cody - Psychology

This research investigates a mode of biased information processing that may characterize social anxiety - a lack of integration between global and local levels of processing (seeing the forest vs. the trees). Addressing this lack of integration may help break the vicious cycle whereby socially anxious people pay close attention to social details when anxious but then fail to use that information to modify their maladaptive self-concept. Difficulty integrating different types of performance feedback may contribute to this negatively biased self-perception. For example, Rapee and Lim (1992) found that individuals with social anxiety evaluated their performance more negatively than objective observers did regarding global indicators of good public speaking (e.g., "generally spoke well") but agreed with observers for specific indicators (e.g., "maintained eye contact"). Even when these individuals were accurate in their perceptions of specific details of their own performance, they seemed to ignore this information when forming an overall impression. The lack of integration between global and local levels of processing may explain why people with social anxiety often do not benefit from corrective social feedback. Instead, they seem to rely on a pre-existing negative schema (cognitive framework) in forming beliefs about themselves.

To investigate the effects of miscalibration between global and local levels of processing, we tested memory for different types of information about one's social performance. We are interested in whether participants high or low in social anxiety differ in their ability to integrate specific performance details (local information) into "accurate" global impressions. Participants high ($n = 45$) or low ($n = 48$) in social anxiety symptoms gave a series of brief impromptu speeches, rated their speaking performance on items reflecting specific behaviors and global impressions, and received standardized feedback from an experimenter on their performance. The false feedback contained only specific details. After receiving the feedback, participants were asked to complete a questionnaire asking how they thought the experimenter would rate their performance (specific behaviors as well as global impressions) based on the oral feedback the experimenter had provided. Participants completed the same questionnaire again after three days, in addition to a measure of the degree to which they ruminated about their speech performance.

Overall, the results of this study suggest that, as expected, participants high in social anxiety rated themselves as worse than those low in social anxiety on global and local items at both time points. Interestingly, socially anxious participants rated global items more negatively than local items, but only after the delay, a relationship that was partially mediated by negative rumination. Participants low in social anxiety did not show this change over time and, moreover, actually rated global items more positively than local items. Therefore, as hypothesized, individuals high or low in social anxiety do seem to differ in their ability to form positive global impressions and maintain them over time, even when given identical local information (specific feedback about their speech performance).

Warning, Objects May Seem Closer Than They Appear: Explicit Preference and Distance

Jennifer Joy-Gaba - Psychology

Perception research has shown your ability to act in the environment can influence individuals' visual perception. For example, wearing a heavy backpack can make a hill appear steeper (Bhalla & Proffitt, 1999), while distance judgments can be influenced by having an intention to walk (Witt et. al, 2004). These results suggest that visual perception is not just based on the geometry of the world, and perhaps other phenomenon might also influence individual perception. In the current research, we examined whether individual preference for an object influenced apparent distance. Using a visual matching task, participants estimated the distance to a Pepsi can at 40, 50, and 60 centimeters. Afterwards, participants completed several questions regarding their preference for Coke and Pepsi. Results indicated that participants who reported a strong Coke preference on a pre-test measure judged the Pepsi can to be closer than participants who had reported preferring Pepsi, $F(1, 67) = 24.19, p < .001, \eta^2 = .27$. Similar results were found for participants preferring Coke in a follow-up questionnaire, $F(1, 67) = 17.11, p < .001, \eta^2 = .20$. Together, these results suggest desirability of an object affects its apparent distance. Specifically, the less desirable an object is, the closer it appears.

Evaluation of Oral Mucositis in the Adult Oncology Population

Jessica Keim - Nursing

Introduction

A patient's journey with cancer is a unique and varied experience that often results in negative side effects of treatment. Oral mucositis is one of the most common and troublesome adverse symptoms experienced by individuals with cancer (Beck, 1996). Even though it is highly bothersome to the patient, mucositis, especially in its earliest stages, is often underreported by clinicians because patients experience a multitude of other symptoms related to cancer. The goal of this project was to determine the overall prevalence of mucositis in the University of Virginia Health System.

Methods

The project utilized a two-pronged approach to understand the problem of clinical mucositis on both a macrosystem and microsystem level for comparison. First, queries were made in the Clinical Data Repository (CDR) at UVA (a large database holding de-identified clinical and financial patient information) to determine the scope of the problem on a macrosystem scale. Queries were made for the 2006-2007 time frame using the ICD-9 code for mucositis and the medication code for stomatitis cocktail. Then, a random assessment of 72 individuals was performed, using a standardized assessment tool for mucositis, to determine the point prevalence of the population.

Results

When the CDR was queried for actual cases of mucositis (based on ICD-9 code) from 2006-2007, there were only 30 cases for the year on oncology inpatient unit and 17 cases in the oncology ambulatory clinics. Of this group, there were 15 inpatient deaths, 5 of which from septicemia. When the medication 'stomatitis cocktail' was queried for the same time period, 156 cases were noted on oncology inpatient unit and 96 cases in the oncology ambulatory clinics. Stomatitis cocktail is the primary medication given for mucositis, so it can be assumed that each stomatitis cocktail prescription per hospital stay was used to treat one case of mucositis. Additionally, using a convenience sample of 72 adult inpatient and outpatient oncology patients, the point prevalence was determined to be 8.3% of the population experiencing mucositis.

Discussion

The conflicting results may represent a disconnect by the physician and nurse practitioner coding system because the results indicate that the diagnoses of mucositis are underreported in the clinical setting. These findings direct a need of accurate nursing assessment and documentation to ensure that mucositis is being managed appropriately. Nurses hold the key for making an impact in early treatment and amelioration of mucositis through adequate and standardized oral assessments in the oncology population. Nurses can also communicate with other members of the health team to identify early changes in the oral cavity that can be acted on swiftly.

Healthcare Experiences of Zabbaleen Women

Andrea Knopp - Nursing

Despite increased life expectancy and decreased maternal deaths since the 1970s, women continue to experience health inequalities in the Middle East. The women of the Zabbaleen community are a unique population in Cairo, Egypt who share several characteristics known to contribute to inequities in healthcare: gender, low socioeconomic status, low literacy/education levels and cultural norms in a low to middle income country. The research question is: What are the Healthcare Experiences of Zabbaleen Adult Women with HCV in Cairo, Egypt? The primary aim of the study was to increase understanding of health disparities and inequities related to culture, ethnic origin, gender and social economic status from a global perspective. The purpose of this qualitative study of the healthcare experiences of these Middle Eastern women is to gain information useful in developing programs to provide culturally appropriate interventions and improve health education, health resources and health access for vulnerable populations.

The ethnographic study included non-participant observation of women and in-depth semi-structured interviews with a convenience sample of 12 women regarding their healthcare experiences including access to and use of resources.

An Arabic language consent form was given to or read to potential participants who could not read. Formal interviews were conducted in which participants were asked to describe their health care experiences. The interview questions were written in Arabic as well as English and cross-translated for verification prior to the interviews. The interviews were recorded with the conversational translation and the translator in Cairo made the transcripts from the recordings. Interview transcriptions were checked for accuracy by reviewing the tape recording with the transcript. Audio tapes in Arabic were translated into audio tapes in English and written text in Arabic and English. The text format was imported into the qualitative program NVIVO 7 to be analyzed. Observations made at the participants' homes and other places where the participants might invite the researcher were included in the field notes.

Preliminary results show three main themes of the healthcare experiences of the women: Fatalism, Fate and Suffering. Several of the women were diagnosed with Hepatitis C (HCV) and related their healthcare experiences in the context of having HCV. These themes present the connections between gender, low socioeconomic status, low literacy/education levels and cultural norms in relation to the women and their health.

From these themes, programs to address areas of health concerns can be developed to address the underserved status of the women in relation to their health situation. Further research needs to be conducted to investigate comparative themes in other communities with similar issues.

Effects of Uncertainty and Unexpectedness on Positive Mood

Dieynaba Ndiaye – Psychology

This study examines factors that can influence people's emotional reactions to positive events. Specifically we investigated the role of expectations and uncertainty about how the event happened. Unexpected events might intensify positive feelings because they create a pleasant surprise. Uncertain events might intensify positive feelings because they motivate people to think about the event, which keeps feelings alive.

Method: 72 University of Virginia undergraduates played a computer game which was allegedly testing their implicit learning skills. This was a 2 Expectations (expected vs. unexpected) x 2 levels of Certainty (certain vs. uncertain) design. Halfway through the game, participants in the expected condition were told that they were doing well at the computer game; in the unexpected condition, participants were told that they were doing poorly. Then all participants received a high score of 94 out of 100. In the certain condition, participants were told that they did well because they used a particular problem solving strategy. In the uncertain condition, participants were told that they would not find out which of two problem solving strategies helped them do well.

Results and Discussion: Only the Expectation x Certainty interaction was significant, $p = .04$. Participants were happiest in the uncertain expected condition ($M = 16.6$), and in the certain unexpected condition ($M = 16.5$). Paradoxically participants who were in the uncertain unexpected condition were less happy ($M = 15.9$), they did not differ from participants in the expected certain condition. These results suggest that an event being EITHER uncertain or unexpected but not both intensifies positive feelings. There was no benefit to feelings when the event was both uncertain and unexpected. In fact, positive feelings were lower. Future research will investigate the hypothesis that a combination of uncertainty and unexpected leads to too much uncertainty about an event, thus becomes negative.

Constructing the Theory of Reasoned Action Waterpipe Questionnaire

Devon Noonan - Nursing

Background - Smoking tobacco using a waterpipe is a new trend among college students and is associated with multiple health problems, including addiction. There are currently very few instruments available to measure waterpipe use among college students. This information is necessary to identify and understand factors affecting waterpipe use in this population. The purpose of this two part study was to develop and perform preliminary psychometric testing on the Theory of Reasoned Action (TRA) Waterpipe Questionnaire (TRA-WQ), an instrument developed to examine psychosocial variables of the Theory of Reasoned Action (TRA) in regard to waterpipe use.

Design/Methods – A two method approach was used for the development and testing of this instrument. A convenience sample (n=59) of students was recruited from undergraduate summer classes at the University of Virginia to participate in the first phase of the study. This phase examined the attitudes and beliefs associated with waterpipe smoking using an open-ended questionnaire based on the TRA to gather data. Content analysis was performed to determine the salient beliefs related to waterpipe smoking in this sample and these findings were used to develop the questions of the TRA-WQ. The second phase of this study assessed the Internal consistency on the scales of the TRA-WQ. A convenient sample of 100 students was recruited at the University of Virginia. Using Cronbach's alpha reliability coefficients the internal

consistency of the scales used in the TRA -WQ was examined.

Results – The mean age of the first sample was 19.8 years. Among the sample 13.8% reported "current" (in the past 30 days) waterpipe use, while 44.8% of the sample reported "ever" waterpipe use (have you ever tried smoking tobacco using a waterpipe even one or two puffs). Salient behavioral beliefs that emerged from the analysis included, "waterpipe smoking is safer than regular cigarettes" and "it's relaxing", "tastes" and "smells" good, "more socially acceptable than cigarette smoking", and it "is a fun way to socialize with friends". These phrases were used to develop the 37-item TRA-WQ based on the Theory of Reasoned Action. The mean age of the second sample was 19.9 years. Among the sample 11.1% reported "current" (in the past 30 days) waterpipe use, while 54% of the sample reported ever waterpipe use (have you ever tried smoking tobacco using a waterpipe even one or two puffs). Reliability coefficients for TRA-WQ variables ranged from .76 to .95.

Conclusion – This instrument will be used to examine psychosocial variables in regard to waterpipe use in the college student population in future studies.

"Fool Me Once, Shame on You. Fool Me Twice, Shame on Me!": Losing and Regaining Trust in Pointing Gestures

Carolyn Palmquist - Psychology

Children seem to expect social cues, like pointing gestures, to be informative (Csibra & Gergely, 2006). Very young children sometimes weight information provided by another person more heavily than their own, independently acquired beliefs. For example, 24-month-olds who see a cat-like animal have the reasonable expectation that it is a cat and will drink milk. However, if they hear an adult refer to it as a "dog," they will infer that it is a dog and that it chews on bones (Jaswal & Markman, 2007). Here, we ask how 24-month-olds resolve a conflict between a salient social cue to an object's location and a salient non-social cue to its location. Specifically, if a buzzing toy is hidden in one of two boxes, and a researcher points toward the empty box, where will children search when invited to find the toy?

For four noisy trials, the researcher hid a wind-up toy in one of two boxes. In the experimental condition (n=17), as she invited children to search and until they made a selection, she pointed to the empty box and alternated gaze between the children and that box. In the control condition (n=6), the researcher invited children to search but did not point toward either box.

On the first trial, 71% of children in the experimental condition followed the deceptive point and searched in the empty box. In the control condition where the experimenter did not point, none of the children searched in the empty box. Thus, on the first trial, children in the experimental condition trusted a social cue indicating the hidden object's location even though it conflicted with a salient non-social cue. Interestingly, having been misled once, these children quickly lost trust in the experimenter's point: on trials 2-4, they searched in the empty box to which she was pointing, on average, just 31% of the time.

Next, children were given four silent trials in which the experimenter hid a silent toy in one of two boxes. In the experimental condition, she pointed at the box in which the toy was hidden, and in the control condition, she simply invited children to search. Would children who had lost trust in the experimenter's pointing gesture regain that trust given that it was now accurate and the only cue to the object's location?

Children in the control condition who had no cues as to the object's location searched randomly across the four trials. Children in the experimental condition who saw the experimenter point to the correct box seemed to re-gain trust in the pointing: Only about half of the children (59%) found the object on the first trial, but on the last three trials, they found it on average 73% of the time.

In summary, while toddlers sometimes weight a social cue, like pointing, more heavily than a salient non-social cue, they can lose their trust in such gestures when they are deceptive. However, children are able to quickly regain trust in a previously incorrect social cue in a situation where it is all that is available.

Thriving with a Plural Self-concept: Relationships among Role Identities Predict Social and Psychological Health

Jesse Pappas - Psychology

Fragmentation models of self-plurality (Donahue, Robins, Roberts, & John, 1993; McReynolds, Altrocci, & House, 2000) interpret high degrees of complexity within the self-concept as a threat to psychological health. Specialization models (Linville, 1987; Rappoport, Baumgartner, & Boone, 1999), however, predict positive psychological outcomes for those with a particularly diverse or plural sense of self. We believe that an important, unexplored area within this domain concerns the quality of interactions among an individual's unique set of social role identities.

Within multiple regression models, new measures of identity plurality, identity harmony, and identity independence were related to indicators of social self-efficacy, subjective well-being, and mental health. Results show that functional harmony among identities within the self-concept is associated with social and psychological vigor. Self-plurality facilitated social confidence, but did not predict well-being or mental health. Like most organized systems, the performance of the self-concept system depends on how well its component parts function as a dynamic unit.

"Guilt-by-Association: Implicit Attitude Generalization Occurs Immediately,"

Kate Ranganath - Psychology

Modern legal thought and moral reasoning largely reject the idea of guilt-by-association (Banaji & Bhaskar, 2000). Most believe that people should be held accountable for their own actions, not for the actions of others (Leventhal, 1980). However, everyday thinking and judging provide occasions in which the actions of one are used in evaluating another without necessarily having conscious intention to do so. In the present research we investigate the possibility that, over time, evaluations of one individual may generalize to other individuals who share group membership, despite conscious intentions to the contrary. Dual-process models distinguish between associative (implicit) and propositional (explicit) forms of information processing (Gawronski & Bodenhausen, 2006). While propositional reasoning determines whether a given thought will be explicitly endorsed, an associative pattern of activation occurs whether or not the individual considers those associations to be true or not. We hypothesized that the mere fact that two individuals are associated at all would be enough for implicit evaluations of one to immediately associatively generalize to another who shares group membership. On the other hand, we hypothesized that explicit attitudes would resist generalization because one could apply a rule that evaluations of one group member do not necessarily apply to other group members. To test this, participants completed an attitude induction procedure in which they read about behaviors performed by two individuals who were members of distinct social groups. One of the individuals performed primarily positive behaviors, the other primarily negative behaviors. Next, participants read brief introductions to two new individuals belonging to the same social groups. The information about the new individuals was minimal and contained an equal amount of positive and negative information about both. As expected, the just-formed explicit attitudes toward the original individuals did not completely generalize to the new group members, but implicit attitudes did. Participants deliberately refrained from using information about some group members in judgments of others from the same group, but were unable to prevent automatic associative generalization. We also examined the stability of attitude generalization over time. We hypothesized that as time passed and forgetting occurred, the conscious ability to distinguish between group members would decrease, leaving explicit evaluations reliant on associative memory. This would result in explicit evaluations that generalize in the long-term the same way that implicit evaluations did immediately. This hypothesis was supported, pointing to the importance of automatic associations in conscious judgment when propositional information is unavailable. Given only a minimal link between individuals, implicit evaluations of a novel individual immediately generalize associatively to other members of the same group. Though people explicitly resist generalization in the short term, over time explicit evaluations generalize, too. Together these results suggest that although people may explicitly endorse values like fairness or egalitarianism, our implicit minds might not always allow us to act on them.

Implicit and Explicit Measures of Peer Rejection as Differential Predictors of Future Adjustment

Megan Schad - Psychology

Youth who are disliked by their peers are well-known to be at increased risk for poorer future adjustment (Parker & Asher, 1987). This study examined the predictive utility of implicit (IPR) versus explicit (EPR) self-perceptions of peer rejection on adolescents' adjustment and interpersonal relationships one year later. Participants were 184 adolescents (ages: T1 M=18.33; T2 M=19.64), along with their best friends (age T2 M=19.39), and romantic partners (age T2 M=18.30). At Time 1 only target adolescents were assessed. At Time 2, adolescents, their best friends, and romantic partners participated in data collection. At Time 1, adolescents' IPR and EPR were assessed using the Implicit Association Test (IAT; Greenwald, McGhee, & Schwartz, 1998) and social acceptance scale from the Self-Perception Profile (Harter, 1988), respectively. At Time 2, adolescents self-reported on their attention problems, anxiety and depression, aggression, and withdrawal using the Adult Self-Report (Achenbach & Rescorla, 2003). Also at Time 2, best friends reported on conflict & betrayal in their friendship using the Friendship Quality Questionnaire (Parker & Asher, 1993) and romantic partners reported on their own positive communication using the Conflict In Relationships Questionnaire (Wolfe, et al. 1994). Results of hierarchical multiple regression analyses suggested that higher levels of IPR predicted greater betrayal and conflict in friendships ($\beta = .26$; $p < .01$) and less use of positive communication strategies in romantic relationships ($\beta = -.29$; $p < .05$) one year later. By contrast, adolescents' EPR predicted higher levels of self-reported anxious and depressive symptoms ($\beta = .39$; $p < .001$), aggression ($\beta = .25$; $p < .01$), withdrawal ($\beta = .34$; $p < .001$), and attention problems ($\beta = .25$; $p < .01$) one year later. Relationship quality (as reported by friends and partners) was predicted by IPR, whereas self-reported adjustment was uniquely predicted by EPR. These results suggest that IPR & EPR may be differential predictors of interpersonal functioning and adjustment.

Peer Quality and Inappropriate Photos on Facebook and MySpace Predict Changes in Young Adults' Psychopathology

David Szwedo - Psychology

Despite the increasing popularity of social-networking websites such as Facebook and MySpace, little is known about how young adults' use of these websites may be related to their psychological well-being over time. For this study, we directly observed: 1) the size of young adults' friendship networks on these sites, 2) the quality of the posted comments they received from peers, and 3) the behaviors exhibited in their posted photos in order to determine whether these qualities predicted a change in levels of anxious/depressive symptoms, social withdrawal, and alcohol use in a one-year longitudinal design.

After controlling for how socially accepted participants perceived themselves to be, having a larger friendship network was associated with a decrease in anxious/depressive symptoms one year later, particularly when youth did not perceive themselves as accepted by others. Having a larger friendship network was also related to an increase in social withdrawal behavior, though receiving supportive comments from friends predicted a decrease in social withdrawal.

Participants' inappropriate behavior in posted pictures, including explicit alcohol use and provocative dress, was associated with an increase in alcohol use and alcohol-related problem behaviors one year later.

These results suggest that feeling connected to a larger number of friends online may protect against negative feelings typically experienced in the context of social rejection, though they may not necessarily lead to greater in-person interaction. Supportive comments from friends, however, may indeed increase social interaction with others. Finally, the presence of inappropriate behavior in pictures was associated with an increase in alcohol-related problems, suggesting there may be some validity to using these pictures as markers of future behavior.

Young Mothers' Feeding Practices

Mary Thompson - Nursing

Purpose

The purpose of this qualitative study is to explore the feeding practices of low-income, teen mothers of preschool-age children. This study uses an innovative method of self-video documentation, where mothers video tape themselves while engaged in activities related to feeding their child. Self-video documentation allows for a better understanding of the factors involved in the preschool child's feeding environment. Parental feeding practices are important to study as research has supported the theory that parental feeding practices can be a risk factor for some children who develop childhood overweight. Childhood overweight is a growing, national, public-health problem.

Sample

Mothers who were teens at the birth of their child were targeted for this study because a) children born to teen parents are at increased risk for health problems; and b) previous research using self-video has been successful with this age group. The preschool-age group was targeted because this is an important time for prevention and intervention for childhood obesity as preschool-age children are in the early stages of developing health habits.

Research Question

What are the feeding practices of low-income mothers who were teens at the birth of their preschool child?

Methods

The data for this study are self-made videos that mothers make during routine meal times over a two week period. In addition, interviews are conducted with the mothers, while viewing the videotapes, to gain a better understanding of what was happening during the filming. This technique provides an ability to study a wide variety of factors that might have an effect on the teen mothers' food choices and feeding practices. The methods are based loosely on a "visual anthropology" and use a phenomenological approach to data analysis. Data management and analysis is aided with the help of NVivo 8 qualitative data analysis computer software.

Findings and Discussion

Data collection and analysis is currently ongoing. Preliminary findings relate multiple factors to the quality of the feeding environment that includes: stress, lack of time and food choices - that are directly related to governmental resources such as food stamps and Medicaid, economics, proximity to grocery stores and healthy food choices for families living in both rural and urban areas and the family social milieu around meal times.

Thermokars Depressions in the Southern Highlands of Mars

Adam Bentham - Environmental Science

Recent missions to Mars have produced a great number of high resolution satellite photographs and have provided images to cover a significant portion of the planet's surface. This allows the systematic investigation of many meso-scale features which were previously irresolvable.

Utilizing images from MRO HiRISE, CTX, THEMIS VIS, and MGS MOC we have identified a latitude-dependent type of depression found on many craters which we are calling Elevated Flat Floor Depressions (EFFDs). A detailed survey of these features was conducted across the Southern Highlands of Mars. We have found that EFFDs are characterized by: 1) A restricted geomorphic region with EFFDs located in a latitude band of between 40° and 60° more prevalent in the southern hemisphere but also present in the same latitude band in the northern hemisphere. 2) A distinctive morphology. EFFDs are typically around 1000m in diameter, orientated parallel to the crater rim, have steep, well defined scarps, and are completely enclosed. Depressions lack both evidence of fluvial or creep related inputs and outputs of sediment and are conspicuous due to their unaccountable volume loss. The depressions are also situated on the apex of medium (30km) size crater rims and quickly decrease in frequency as the distance from the rim increases. 3) Recent formations likely Amazonian in age (<1.8 b). EFFDs "fresh" appearance with distinct cliff forming scarps on eroded craters with mantled terrains, evidence of recent back wasting, and dearth of superimposed features indicate that EFFDs post date not only crater formation but most of the erosional period since.

We conclude that the latitude dependence, morphology, and unaccounted volume loss these features indicate that the depressions are periglacial in origin similar to terrestrial thermokarst depressions where subsurface volatile rich deposits are being exposed to the atmosphere. We believe that crater rims create a situation where erosional removal of material is at its maximum and deposition of down slope creep and aeolian sediment is minimal. This erosional regime removes a top, protective layer of material and exposes subsurface layers to atmospheric conditions unstable for ice which then sublimates away.

Shrub and tree expansion in the circumpolar Arctic: spatial analysis of ecological state-shifts since the 1960s

Gerald Frost – Environmental Science

Patterns of tall shrub and tree occurrence form conspicuous and dynamic ecological boundaries across arctic regions. The northward and upslope encroachment of tall shrubs and trees into tundra-dominated areas is one of the principle changes to arctic land cover expected with a warming climate, and there is mounting evidence that ecological state-shifts are already occurring in tundra ecotones of North America. The ubiquity of these state-shifts across the circumpolar arctic is unclear, however, because few data exist for the vast Eurasian continent. This study is documenting recent changes in tundra ecotones at ~25 sites in northern Eurasia and Alaska through visual photo-interpretation of circa 1965 “Corona” and contemporary high-resolution satellite photography.

Corona was the world’s first operational satellite surveillance system and today offers a readily available source of data for land surface change studies conducted across large spatial scales over a ~40 year temporal interval. The degree to which patterns of vegetation change are shared among widely distributed study sites will indicate the ubiquity of ecological state-shifts in the Arctic, as well as the relative influence of large-scale forcing mechanisms (e.g., climatic change) and local environmental controls (e.g., distribution of favorable geomorphic features in the landscape) on shrub and tree expansion in arctic ecosystems. Remote sensing and ground-based data indicate that mean annual temperatures have increased by 1.5 degrees C or more over the last ~half century at all of the study sites.

Preliminary findings indicate that tall shrublands have expanded at several sites in northwestern and far eastern Eurasia, and western Alaska. Findings for Alaska are corroborated by ground-based repeat photographs. The changes observed thus far are unidirectional, with no die-back of shrublands detected at any site. The degree of shrub expansion is highly variable both within and among sites, however, with dramatic expansion at some locations and little change at others. In northeastern Eurasia, tundra ecotones are primarily defined by coniferous woodlands, rather than shrublands. Infilling of pre-existing woodlands and establishment of trees ~0.25 km beyond the 1965 treeline position were detected on the lower Khatanga and Kolyma river floodplains. Large-scale, synchronous shrubland and forest expansions have occurred in the past (e.g., mid-Holocene) and associated changes to land surface-atmosphere interactions can have far-reaching effects on atmospheric circulation and global climate.

Towards the Total Synthesis of (-)-Uprolide D

Celine Griot - Chemistry

(-) Uprolide D, a cembranolide isolated from the gorgonian *Eunicea mammosa* displays cytotoxic activity against HeLa cells ($IC_{50} = 5.0 \mu\text{g/mL} = 0.0143 \mu\text{mol/mL}$). The limited supply (0.001% of extract) of (-)-uprolide D makes it interesting target for synthetic effort.

(-)-Uprolide D contains several challenging structural elements that must be overcome to achieve a total synthesis of this molecule. The cembranolide contains an oxygen bridged 14-membered ring which constitutes the core of the natural product, an embedded tetrahydrofuran ring as well as a fused γ -lactone. (-)-Uprolide D also possesses seven stereocenters, one of which is quaternary which adds an additional element of difficulty to the synthesis due to the complexity and sensitivity of the system. To date, no total synthesis of (-)-uprolide D has been reported.

A plan was developed for the total synthesis of (-)-uprolide D utilizing a convergent synthesis. One of the key steps would be the simultaneous formation of the macrocycle and the fused γ -lactone.

A spatially-explicit model of flow routing and lake extent: Application to modern and late Pleistocene lakes in the U.S. Great Basin

Yo Matsubara – Environmental Science

In most terrestrial fluvial networks, storage and evaporative loss of water in lakes and reservoirs have negligible impact on water budgets. However, in some landscapes, such as the U.S. Great Basin region, climate changes cause large variation in the number and size of lakes as well as the connectivity and magnitude of inter-basin flows. Conversely, changes in lake level in enclosed basins preserved over geologic time provide information on the local climate change.

A spatially-explicit hydrological model was applied to the Great Basin region in the western United States to predict runoff magnitude and lake distributions under modern and late Pleistocene conditions (20-10 ka). The model iteratively routes runoff through depression to find a steady state solution, and was calibrated with annual precipitation, pan evaporation, mean annual temperature (MAT), and yearly stream runoff. This model is not coupled with an atmospheric model. Rather, this exploratory study focuses on testing of the flow routing model and determining what changes in climatic factors relative to the present conditions were required to create the Pleistocene mega-lakes based on a simple balance between precipitation and evaporation.

The model was first validated by applying it to the present day Great Basin. Then each equation was scaled accordingly to simulate the paleo-conditions. The predicted lake distribution provides a close match to present-day lakes. For the late Pleistocene time, the sizes of lakes Bonneville and Lahontan are well predicted by linear combinations of 0.2 to 5.8 °C decreases in mean annual temperature from present day and increases in precipitation from 1 to 2 times modern values. This corresponds to runoff depths ranging from 1.8 to 4.4 times the present values and yearly evaporation from 0.4 to 1 times modern values. To reproduce Lake Manly in Death Valley, however, combinations of temperature decrease up to 9 °C or precipitation up to 2.4 times the present values were required. Possible explanations for this latitudinal difference in estimated climate include groundwater contributions to Lake Manly or spatially non-uniform climate due to topography.

A 3-Liter Capacity ³He Polarizer for Medical Imaging

Karen Mooney - Physics

Hyperpolarized ³He gas has been used in Magnetic Resonance Imaging (MRI) for over a decade, producing high quality lung images in a variety of applications. The original ³He polarizers built for medical use are capable of producing 1L of ³He per day at up to 40% polarization, by using collisional spin exchange with an optically pumped rubidium vapor. Recent technical developments such as hybrid spin-exchange optical pumping (SEOP) and commercially available high-power line-narrowed diode lasers offer the promise of higher ³He polarization, shorter time to reach maximum polarization, and larger production volumes. These developments have been used to improve ³He targets for nuclear accelerator experiments over the past several years. It was the goal of our group to bring these advancements to the medical field and enhance polarizer performance. The result is a device that produces 3L of ³He per day at 60% polarization. This new ³He polarizer will be used for lung imaging research in the Radiology Department at UVa.

"Simulating Soil Organic Nitrogen and Grazing Effects on Arctic Tundra Vegetation Dynamics on the Yamal Peninsula, Russia"

Qin Yu – Environmental Science

Sustainability of tundra vegetation on the Yamal Peninsula, home to the world's largest area of reindeer husbandry, under changing climate is of crucial importance to the local native community. In this study we applied a nutrient-based plant community model (ArcVeg) to evaluate the dynamics of tundra ecosystems on the Yamal Peninsula with different soils (both sandy and clayey soils with different soil organic nitrogen levels) and different grazing regimes across a latitudinal climatic gradient. We examined how these three factors (climate zone, soil organic nitrogen levels, and grazing) interact to affect tundra response to climate warming. Model simulations were driven by field-collected soil data and observed grazing patterns along the Yamal Arctic Transect (YAT). Soil data for both sand and clay sites within bioclimate subzones C (High Arctic), D (northern Low Arctic) and E (southern Low Arctic) were used. Model simulations demonstrate how climate zones, soils, grazing, and warming can interact and affect tundra plant biomass. Nutrient poor soils (i.e. low soil organic nitrogen or SON) support not surprisingly less vegetation than nutrient rich soils. Soil organic nitrogen may not only be a limiting factor to plant growth, but also may limit plant responses to climate change. Soil organic nitrogen interacts with climate zone to affect Net Primary Productivity (NPP). In already nutrient poor sites, grazing may not have a direct effect on NPP (sandy sites in Laborovaya LV-2 and Karasavey KH-1). This study improves our understanding of how soils, climate, and grazing can interact and drive tundra vegetation in the future.

The reactivity and oxygenation of tungsten phenol systems

Victor Zottig - Chemistry

Aromatic compounds are abundant, readily available, and inexpensive, many of which can be found in crude oil. Aromatic molecules, although abundant are not especially useful compounds for materials or products used in every day life. The inherent aromatic stabilization of the molecules prevents widespread use as precursors for organic reactions to product biologically active or pharmaceutically useful products. Phenol (benzene with an alcohol group on the molecule) and phenol derivatives are aromatic molecules found in substances such as poison ivy, aspirin, and in the amino acid tyrosine. The pharmaceutical and drug industry would be interested in the oxygenation of phenol, which eliminates the aromaticity and forms compounds similar to sugars, carbohydrates, and vitamin B8.

Considerable attention has been devoted to the study of aromatic molecule activation via coordination to metal complexes. The coordination of a metal complex to an aromatic molecule can augment the reactivity of the molecule and thus make it susceptible to traditional organic reactions. The Harman lab has been dedicated to the fundamental study of using transition metals, particularly tungsten, to break the aromatic stability in order to undergo organic reactions. By coordination of phenol to the tungsten metal fragment, we can tightly control both the geometry and placement of oxygen functional groups on the former aromatic ring. Coordination enables us to potentially synthesize biologically active molecules from an aromatic compound.

The goal of this study is to extend the range of novel reactions to include hydroxylations, epoxidations, and other oxygenation reactions. While formation and characterization of the epoxide from the parent phenol has been elusive, the stereochemistry (geometry) and regiochemistry (placement) of products is consistent with an epoxide intermediate. By replacing phenol with a simple derivative, m-cresol the epoxide could be isolated and characterized.

Chromodomain recognition of epigenetic marks

Bartłomiej Blus - Computational Biology & Biophysics

The post-translational modification of specific lysines along histone H3 tail regulates gene expression through the recruitment of specialized chromatin factors that contain chromodomains. To better understand epigenetic signaling, it is important to delineate the rules that dictate the docking interactions between recognition modules and modified histone tails. Here, we provide evidence that two distinct mechanisms are used by LHP1 and CHD1 chromodomains to recognize methyllysines 27 and 4 in histone H3 tail, respectively. Lysine 27 methylation contributes to gene repression during development, whereas lysine 4 methylation promotes transcription activity. A single chromodomain in LHP1 protein forms a 1:1 interaction with the region of histone H3 tail surrounding methyllysine 27. This methyllysine resides within an ARKS motif which forms complementary interaction with the surface of the chromodomain. The methylammonium group is captured via cation- π bonding by an aromatic cage involving three conserved aromatic residues in LHP1. Interestingly, the ARKS motif also surrounds methyllysine 9 in histone H3 tail. In vitro studies support ambivalent binding interaction between LHP1 and methyllysines 9 and 27 in H3 tail. However, in vivo characterizations support a dominant presence of LHP1 at chromatin with methyllysine 27. Unlike LHP1, CHD1 protein contains tandem chromodomains that assemble cooperatively to bind a lysine-methylated peptide. The structures of the CHD1 tandem chromodomains in the free and peptide bound states show a substantial difference with LHP1 chromodomain. Our studies provide important insights about methyllysine recognition by single and double chromodomains, and help predict function of uncharacterized chromodomains.

Identification of transcription factors that regulate nicotine biosynthesis in tobacco using a novel pulldown assay

Marta Bokowiec - Biology

Along with morphine, caffeine and codeine, nicotine is one of the most important secondary metabolites in plants. In tobacco (*Nicotiana tabacum* L.), nicotine is an anti-herbivore compound and is induced by both wounding by herbivores and the plant hormone jasmonic acid (JA). Given the importance of nicotine, it is surprising that little is known about the regulation of nicotine production. The first committed step of nicotine biosynthesis is catalyzed by the enzyme putrescine N methyltransferase (PMT). PMT is encoded by a small gene family of five genes (PMT1a, -1b, -2, -3, -4) in tobacco. PMT gene expression is induced by wounding and JA and transcription of the NtPMT1a gene is rapidly induced in tobacco Bright Yellow-2 (BY-2) cell cultures following methyl jasmonate (MeJA) treatment. Within the NtPMT promoters, I have characterized a novel tripartite motif, termed the GAG motif, consisting of a G box-like element, an AT-rich region, and a GCC box-like element. This GAG motif functions as a unit and is required for MeJA-induced NtPMT1a transcription. Analysis in tobacco plants showed that GAG motif tetramers directed expression in root cortical cells following treatment by MeJA and leaf wounding. My analysis of the GAG motif suggests that it is the target of several classes of transcription factors. The involvement of three motifs suggests the formation of complexes across the GAG motif. However, little is known about the protein complexes that regulate transcription in plants, partly due to the lack of available methods. My goal was therefore to develop a biotin – streptavidin pulldown assay for the identification of protein complexes from plant promoters. This method uses nuclear protein extract from tobacco BY-2 cells and defined promoter elements. Protein complexes that form on the promoter are pulled down and then separated on SDS-PAGE gels and analyzed by LC-MS/MS. Using this method, I have successfully detected specific binding to different promoter elements. Several proteins were found to bind to the GAG motif, and mutations that abolish function of the GAG motif in planta result in the loss of at least five major proteins. This loss of binding correlates with loss of function and suggests that these proteins are components of complexes that regulate transcription from the GAG motif. This is a significant new tool for the study of transcriptional regulation in plants.

CD8 T cells activated in different lymph nodes distribute systemically, express homing molecules programmed in the original sites of activation in the steady state, and exhibit varying levels of plasticity

Colin Brinkman - Microbiology

Using localized dendritic cell immunization, we have previously demonstrated that a significant fraction of divided CD8 T cells redistribute into Ag-free lymph nodes (LN) within 3 d of activation. CD8 T cells that redistribute exhibit characteristics of fully differentiated effectors (IFN-gamma production, CD127neg) comparable to splenic effectors. These cells also contain memory precursors.

We have now examined the expression of homing receptor molecules on cells in the initial activation site and after redistribution to Ag-free LN. alpha4beta1 integrin was induced on CD8 T cells activated in mesenteric and mediastinal LNs and was retained after they redistributed into Ag-free axillary LN. Similarly, the 1B11 epitope of CD43 and E-selectin ligand were induced on CD8 T cells activated in axillary LN and were retained after T cells redistributed into Ag-free mesenteric and mediastinal lymph nodes. These redistributed cells also did not acquire “new” homing molecules characteristic of the sites into which they migrated.

Additionally, homing molecules induced during activation were maintained on resting memory cells. alpha4beta1+ memory cells primed in mediastinal LN and spleen distributed systemically, while 1B11/ESL+ memory cells primed in cutaneous LN preferentially resided in cutaneous LN. Preliminary results suggest that this programming persists during the recall response for alpha4beta1+ cells and prevents upregulation of 1B11 and ESL upon reactivation in cutaneous LN. This phenomenon may explain the differential control of tumors growing in different parts of the body by memory cells induced through dendritic cell vaccination by different routes (Mullins et al., JEM, 198, 1023).

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Signaling to Chromatin: The Role of Histone H3 Methylation in STAT-Dependent Gene Expression

Lauren Buro - Biology

STATs are a family of transcription factors that, in response to a variety of extracellular ligands, are rapidly and transiently recruited from their latent state in the cytoplasm to the nucleus where they drive transcription of target genes. The accessibility of the activated STAT to its DNA binding site and the succeeding gene expression are intimately tied to chromatin structure. Post-translational modifications of the residues on the amino terminal tails of the lysine residues of histones define the functional state of the chromatin. Methylation of lysine residues of Histone H3 (H3) is described as either activating or repressive of gene expression, with the activity correlated to the position of the lysine residue (K4, K9 or K27), the level of methylation (mono, di or trimethylation) and the location of the nucleosome itself along the gene. Chromatin immunoprecipitation followed by real-time PCR demonstrate H3K4 dimethylation (H3K4 me2) and H3K4 trimethylation (H3K4 me3) primarily in the control regions of all Interferon gamma inducible genes assayed, independent of transcriptional activity. When these genes are induced, however, both H3K4 me2 and me3 increase. Chemical inhibition of methyltransferase activity decreases both transcription as well as H3K4 me3 levels. When demethylase activity is chemically inhibited, however, no effect is observed on transcriptional activity or H3K4 me2 and me3 levels on the IRF1 gene. These results support the idea that H3K4 me2 and me3 play a dynamic and functional role in gene expression of STAT-dependent gene loci. Experiments to identify the methyltransferase as well as determining the function of the demethylase are ongoing.

Combining Multiple Tract Tracing, Confocal and Electron Microscopy: Visualization of Multiple Primary Afferents Contacting Individual Projection Neurons

James Corson - Psychology

Determining the circuitry of a sensory system can be a complicated task, especially when that system receives multiple primary inputs. The rostral portion of the nucleus of the solitary tract (NTS) receives inputs from the chorda tympani, greater superficial petrosal, and glossopharyngeal nerves, making it a suitable system to develop a methodology to study the selectivity of individual inputs to postsynaptic cells. We have optimized a method using multiple tract tracing, confocal, and electron microscopy for combining wide scale morphometry, afferent circuitry elucidation, and focal ultrastructural analysis. The chorda tympani and glossopharyngeal nerves were anterogradely labeled with tetramethylrhodamine and Cy5 respectively. In addition, projection neurons in the NTS were retrogradely filled by extracellular injection of biotinylated dextran amine (BDA) into parabrachial nucleus. After two days survival, brains were fixed and the NTS sectioned horizontally. Sections were treated with streptavidin conjugated to Alexa488 to visualize BDA. NTS sections containing anterograde projection fields of both nerves and the retrogradely-filled projection neurons were scanned on an Olympus IX70 equipped with a Fluoview 3.3 confocal laser scanning system with a 60x, 1.4 N.A, oil immersion objective and a 1.5x optical zoom. Z-step resolution was set at 150nm. Confocal image stacks of retrogradely filled cells were then reconstructed in three-dimensions after noise removal and fluorescent brightness optimization using the 3-D imaging software Velocity. Closely apposing processes were identified in the reconstructions as putative synaptic connections. The tissue was then incubated in antibodies for the fluorophores, treated for an avidin-biotin diaminobenzidine reaction, and processed for electron microscopy using osmium fixation and Embed-812 resin embedding. Dendrites imaged on the confocal microscope were then isolated and re-sectioned on an ultramicrotome for serial electron microscopy. The tissue was then examined on a JEOL 1010 transmission electron microscope. This technique allows for the visualization of multiple inputs contacting identified neurons and the ability to study the distribution of those inputs along a cell's dendritic arborization, determining any segregation or convergence of the afferents.

The Adaptor Protein Shc Plays a Key Role in Early B Cell Development

Amber Deusterman - Microbiology

B cell development is a highly ordered process that requires immature cells to pass through multiple checkpoints to advance through development. Successful expression and productive signaling via antigen and cytokine receptors are required at specific stages of development. The adaptor protein Shc is phosphorylated on three critical tyrosine residues and facilitates signaling downstream of multiple receptors, including the pre-T cell receptor. However, its role in B cell development is currently unknown.

Here, using a Cre-loxP mediated approach, we inducibly expressed as transgenes either wild type Shc or a mutant form Shc (ShcFFF, where three critical tyrosines have been mutated). Ig α -Cre-mediated induction of ShcFFF expression (but not wt Shc) at the common lymphoid progenitor stage resulted in a severe block at the pre-pro-B to pro-B transition, with diminished numbers maintained throughout B development. Expression of ShcFFF at later stages of B cell development (via CD19-Cre) showed no defect in B cell numbers, emphasizing the importance of Shc during early B development. Mechanistically, Shc appears to affect B cell development at the level of cytokine dependent proliferation, as well as pre-BCR signaling. These results suggest a non-redundant role for Shc in early B development.

Specific SNARE-mediated fusion of single synaptobrevin vesicles with planar supported bilayers containing acceptor-SNARE complex

Marta Domanska - Mol Phys&Biol Physics

The rapid fusion of synaptic vesicles during exocytosis at the active zones of the neuronal plasma membrane has been extensively studied in cells and reconstituted model systems. A variety of cytosolic and membrane anchored proteins compose the fusion machinery that is well conserved in animals. SNARE (Soluble N-ethylmaleimide-sensitive factor Attachment Proteins Receptors) proteins play an essential role in this machinery as proven by numerous biological evidences such as SNAREs structural analogy to viral fusion proteins, sensitivity to tetanus and botulinum neurotoxins and the ability to fuse liposomes in vitro. They consist of three evolutionary conserved families: syntaxin1a, SNAP25, localized in the plasma membrane and synaptobrevin2 which resides in the synaptic vesicles membrane.

In vitro fusion assays like liposome or planar supported bilayers fusion assays have the advantage that elementary fusion reaction with minimal essential components can be studied. However, a problem with the liposome assays of this process has been the unphysiologically low rate of fusion encountered. Single vesicle assays with planar membranes allowed the observation of much faster fusion events, but the physiological specificity of these events has not been unambiguously demonstrated yet. Here we present a fluorescence-based single vesicle fusion assay in planar supported bilayers with improved SNARE reconstitution procedures. The acceptor SNARE complex was pre-assembled from syntaxin 1a (Syx1a), SNAP25 and a short soluble synaptobrevin 2 peptide (Syb49-96) at a 1:1:1 stoichiometric ratio. Planar bilayers were prepared by a combined Langmuir-Blodgett – vesicle fusion technique, which preserves the native topology of SNAREs. The specificity of the acceptor SNARE complex in the bilayers was confirmed by binding of labeled, soluble synaptobrevin peptides. Docking and fusion of single Rh-DOPE labeled v-SNARE vesicles to the supported acceptor SNARE membranes were observed by total internal reflection fluorescence microscopy. Membranes with different lipid compositions were tested. V-SNARE docking was dependent on the acceptor SNARE complex density in the supported bilayers and fusion of docked vesicles occurred within tens of milliseconds. Vesicle docking and fusion was SNAP25-dependent, Ca²⁺-independent and heat-independent. No docking or fusion was observed in protein-free control membranes, syntaxin 1a only or Syx1a:Syx1a:SNAP25 complex containing bilayers.

Influences of Competition on Gustatory Development

Sara Dudgeon - Neuroscience

Neural competition among multiple inputs during development can affect the growth and organization of circuits in many sensory systems. We aim to explore the role of competition in development of the rat gustatory nerve terminal fields in the nucleus of the solitary tract. The gustatory system has three distinct and partially overlapping inputs: the greater superficial petrosal (GSP) nerve, the glossopharyngeal (IX) nerve, and the chorda tympani (CT) nerve. The terminal field volume and degree of overlap of these nerves is greatest early in development and decreases as animals age to adulthood. We sectioned the GSP and IX at postnatal day 15 (P15) to assess the effects of lack of competition from these nerves on the development of the CT terminal field. At 35 days post nerve section, a time before GSP and IX regain function to transmit taste information, the CT was labeled with biotinylated dextran amine and subsequently visualized with Streptavidin Alexa Fluor 488. Terminal field volumes were assessed using confocal microscopy and image analysis software. The results show that sectioning the GSP and IX at P15 results in a CT terminal field volume at adulthood much greater than that of control animals. This indicates that the CT terminal field does not reorganize and prune back as it does in normal development when competitive influences from GSP and IX are removed. These studies provide evidence that competition between individual inputs to the gustatory system plays a role in setting up the mature organization of the terminal fields.

RSK2 controls cell survival through spatial restriction of the apoptosis promoting factor, TIA-1, to stress granules

Karin Eisinger - Microbiology

RSK2 is a Ser/Thr kinase downstream of ERK1/2. Until recently, our understanding of RSK2 function has been limited to its role in cell growth and development. We have found a novel, unanticipated link between stress granules and RSK2. RSK2 is a key component of stress granules in MCF-7 cells subjected to oxidative stress (sodium arsenite, 500 mM; 1hr). Stress granules are RNA granules that form in the cytoplasm of cells exposed to various types of stress. Specific mRNAs are stored in the granules and are protected from decay. These mRNAs are available for immediate translation when the stress abates. Both specific knockdown of RSK2 and treatment with the RSK-specific inhibitor, SL0101 (100 mM; 4h), reduce stress granule formation by 75% and 50% respectively in response to oxidative stress. These findings suggest that RSK2 kinase activity is essential for stress granule formation. We have found, by GST-binding assay, that recombinant RSK2 interacts directly with the essential pro-apoptotic stress granule marker, TIA-1. Using RSK2 and TIA-1 deletion mutants we have shown that the N-terminal portion of RSK2, amino acids 1-389, interacts with the C-terminal prion-like domain of TIA-1. Consistent with these findings, we have observed that an N-terminal mutant RSK2, RSK2(1-389), is able to localize to stress granules upon oxidative stress. However, the C-terminal mutant RSK2(373-741) cannot localize to stress granules. These data suggest that the N-terminal domain of RSK2 regulates stress granule formation via its interaction with TIA-1. Additionally, a RSK2 kinase dead mutant, RSK2(K100A), can neither bind TIA-1 nor localize to stress granules. These data are consistent with the observation that inhibition of RSK2 kinase activity decreases stress granule formation. Importantly, decreased RSK2 levels significantly reduced the ability of breast cell lines to survive oxidative stress by increasing apoptosis. Consistent with these data, knockdown of RSK2, by short hairpin RNA, in primary human epithelial cells followed by treatment with sodium arsenite caused an increase in cleaved caspase 3 levels. Together, these data indicate that RSK2 functions in cell survival by facilitating stress granule formation.

Activation of miRNA Processing During *Xenopus* Oocyte Maturation

Alexandra Garcia - Biology

microRNAs (miRNAs) are short, noncoding RNAs that mediate post-transcriptional silencing of target mRNAs in diverse developmental and physiological contexts. miRNAs are among the zygotic genes first activated at the midblastula transition and underlie the maternal–zygotic transition by triggering the degradation of maternal mRNAs. Although expression profiles for several *Xenopus* miRNAs have been reported, the developmental regulation of miRNA processing activities has not been addressed. To this end, we have characterized the regulation of the miRNA maturation pathway in *Xenopus* oocytes. Although miRNAs are zygotically expressed, the protein constituents of the micro-processor complex, Drosha and DGCR8, and the RISC components, Dicer and Ago2, are all maternally expressed. The maternal mRNAs encoding all four proteins are translationally repressed in immature oocytes and are activated late in oogenesis. Although their protein components are present in fully-grown stage VI oocytes, both pri-miRNA and pre-miRNA processing activities are absent until progesterone-induced meiotic maturation. The differential localization of processing activities between the nucleus and cytoplasm of stage VI oocytes may contribute to this repression. Interestingly, although pri-miRNA processing is a "nuclear" event, it is activated only upon breakdown of the nuclear envelope at maturation. Inhibition of either Drosha or DGCR8 expression with antisense morpholino oligonucleotides shows that pri-miRNA processing requires both proteins. Inhibition of Dicer activity inhibits pre-miRNA processing in mature oocytes. We also show that mature oocytes are competent to assemble injected miRNAs into the RISC, even though endogenous miRNAs are not expressed until zygotic gene activation. Our results indicate that oocytes have multiple mechanisms to preclude the generation of functional miRNAs before maturation. These include the rapid degradation of pri- and pre-miRNA substrates, the differential nuclear and cytoplasmic distribution of processing proteins, and translational repression. Our results reveal novel aspects of miRNA processing during oogenesis that differ from what has been characterized in somatic cells.

Modulation of Entorhinal Cortex Neurons by Adenosine and Implications in TLE

Nick Hargus - Neuroscience

Epilepsy is a neurological disorder affecting over two million Americans (approximately 1% of the population) with over 100,000 new cases diagnosed each year. Temporal lobe epilepsy (TLE), the most common form of adult epilepsy, is marked by seizures originating from the temporal lobe structures, most notably the hippocampus. Previous reports have suggested adenosine to be one of the brain's endogenous anticonvulsants because of its ability to inhibit excitatory synaptic transmission. Indeed, adenosine's role as a modulator of synaptic transmission and neuronal excitability has been demonstrated in the CA3 and CA1 regions of the hippocampus. However, it has remained relatively unexplored in the entorhinal cortex (EC), the main input area to the hippocampus, as well as one of the three major output areas of the hippocampus. The EC is thought to play a major role in seizure generation and spread into the hippocampal complex, as such, modulation of excitability of EC neurons could provide an effective way of inhibiting seizure activity.

In the presence of both excitatory and inhibitory blockers of synaptic transmission, non-stellate EC layer II neurons recorded from TLE brain slices were intrinsically hyperexcitable as compared to control neurons. Resting membrane potentials were more depolarized in TLE neurons compared with control neurons. Neurons from the TLE condition also exhibited a reduced action potential threshold as compared to controls, resulting in action potential firing at reduced current injection pulses. When examined at a depolarizing current injection step of 470 pA, a higher frequency of action potential discharges were evoked in TLE neurons compared with control neurons. Upstroke velocity was also increased in TLE neurons. The effects of adenosine were examined on both the membrane properties and synaptically evoked action potentials. In TLE neurons, adenosine at 500 nM and 1 μ M reversibly increased the threshold for action potential generation and reduced the number of synaptically evoked action potentials. In contrast, adenosine at these concentrations had no effect on membrane properties or synaptically evoked action potentials from control neurons. Concentrations as high as 10 μ M were required in control neurons to inhibit action potential firing. Immunohistochemical experiments also showed an increase in adenosine A1 receptor expression in EC layer II neurons from our model of TLE as compared to controls, suggesting that adenosine's action in TLE are more pronounced due to changes in receptor expression levels in TLE neurons.

Increasing the sensitivity of catecholamine detection at carbon fiber microelectrodes by carbon nanotube dip-coating

Christopher Jacobs - Chemistry

Carbon fiber microelectrodes are commonly used for catecholamine detection due to their small size and ease of fabrication. Recent research has shown that coating carbon electrodes with carbon nanotubes can improve electrode sensitivity as well as electron transfer kinetics. In a previous study, we showed that a nafion polymer can be used to suspend and deposit carbon nanotubes on a carbon fiber microelectrode surface. This method however, relies on one second dipping times and was not very reproducible. The goal of the current study was to investigate different solvents for the suspension of carbon nanotubes and to optimize a method that will deposit carbon nanotubes on the surface of microelectrodes. We found that surfactants such as sodium dodecyl sulfate and sodium dodecylbenzenesulfonate suspend carbon nanotubes effectively but lead to electrodes with high noise when used for fast-scan cyclic voltammetry. Dimethylformamide (DMF) was found to be a more effective suspension agent, which led to an increase in sensitivity as well as an increase in the S/N ratio for detection of both dopamine and 2-(3,4-dihydroxyphenyl)acetic acid (DOPAC), an anionic metabolite of dopamine. Increased electron transfer kinetics for the detection of DOPAC were also observed. These studies show that carbon nanotube coatings of carbon fiber microelectrodes increase the sensitivity and electron transfer kinetics for catecholamine detection.

Sirtuin-dependent enhancement of yeast rDNA silencing and replicative lifespan by isonicotinamide

Julie McClure - Cell Biology

Heterochromatic gene silencing in the budding yeast, *Saccharomyces cerevisiae*, occurs at several genomic locations, including telomeres, the HML and HMR loci, and the rDNA tandem array. Silencing requires histone deacetylation by Sir2, the founding member of the Sirtuin family of NAD-dependent protein deacetylases. Sir2 also promotes longevity of this organism by repressing recombination between the rDNA repeats. Nicotinamide (NAM) is a small molecule byproduct of the Sirtuin-mediated deacetylation reaction that also noncompetitively inhibits the reaction when it accumulates to high levels. Isonicotinamide (INAM) activates Sir2 and other Sirtuins by relieving the (NAM) inhibition, thereby resulting in strengthened silencing phenotypes. When monitoring silencing at the rDNA locus (known as "rDNA silencing"), we found that adding INAM to the growth media specifically modifies the epigenetic properties of rDNA silencing such that the silenced chromatin state becomes measurably heritable, a characteristic previously observed at telomeres, but not at the rDNA. We have also found that, along with Sir2, the related Sirtuin, Hst1, is required for stable rDNA silencing under this condition. Hst1 was shown using chromatin immunoprecipitations to specifically interact with the rDNA at the NTS1 region, indicating a novel function for Hst1 in regulating rDNA chromatin. Additionally, we have shown that INAM extends the replicative lifespan of yeast cells in a Sir2-dependent manner by increasing the intracellular NAD concentration. INAM therefore activates Sirtuins through two independent mechanisms, i) relief of NAM inhibition and ii) elevation of NAD levels.

Free radical signaling mediates subunit-specific nitrous oxide inhibition of T-type calcium channels in the pain pathway.

Peihan Orestes – Neuroscience/Anesthesiology

Nitrous oxide (N₂O; laughing gas) has been used as an anesthetic and analgesic for centuries, but its mechanism of action remains unclear. Previous studies have shown that N₂O blocks native T-type Ca²⁺ current in sensory neurons and recombinant CaV3.2 channels. Furthermore, N₂O requires a key metal-binding histidine (H191) in the extracellular IS3-4 loop for its action on CaV3.2 channels.

Here, we show that N₂O-mediated inhibition is also dependent on the presence of common trace metal ions. Diethylenetriamine pentaacetic acid (DTPA), a non-specific metal chelator, effectively reduces N₂O block of calcium current through CaV3.2 channels by 70%. We also implicate free radicals as mediators of this effect since free radical scavengers such as catalase (500U/ml) and α -tocopherol (vitamin E; 100 μ M), are able to reduce the inhibitory effects of N₂O to 7% and 16% of control, respectively.

Thus we hypothesize that N₂O is able to generate free radicals in the presence of trace metal ions, similar to those produced by hydrogen peroxide (H₂O₂). In accordance, we show that N₂O is unable to achieve additional block of CaV3.2 channels over that of H₂O₂. Additionally, we show that N₂O is able to initiate the free radical-mediated oxidation of epinephrine to adrenochrome in the presence of trace metals. The production of adrenochrome was quantified by measurements of absorbance changes at 485nm.

To definitively establish the role of CaV3.2 channels in N₂O analgesia, we injected formalin into the hindpaws of CaV3.2 knockout mice and their wild-type littermates. Following injection, the wild-type mice showed a significant decrease in licking/biting time in the presence of 20% N₂O. In contrast, knockout animals show little decrease in pain with N₂O.

Since recent data strongly suggest the crucial role of Cav3.2 T-type Ca²⁺ channels in pain transmission, N₂O block of these channels mediated by free radical signaling may contribute to part of nitrous oxide's potent analgesic effects.

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CD24 is a target for novel anti-metastatic therapeutics

Jon Overdevest - Molecular Physiology

Nearly half of bladder cancer patients that present with locally advanced disease have occult distant metastases at presentation and poor 5-year survival rate. We recently identified CD24, a small GPI-linked glycoprotein, as necessary for proliferation, anchorage independent growth, and survival of several human bladder cancer cell lines. Additionally, CD24 is an independent prognostic marker for bladder cancer in patients. These characteristics suggest that CD24 may be a good therapeutic target in bladder cancer. Moreover, using our UM-UC-3 bladder cancer metastasis model, we have recently found that in vivo selection of cells tropic to the lungs results in populations of cells with 9.72-fold increased levels of CD24 expression. Given prior reports of the use of anti-CD24 monoclonal antibodies for a post-transplant B-lymphoproliferative disorder, we elected to evaluate the utility of this modality in the setting of our metastasis model.

Briefly, we inoculated nude mice via tail vein injection with these highly metastatic CD24+ UM-UC-3 cells and randomly distributed the mice into three intravenous treatment cohorts: 0.09% saline, 0.1mg IgG1 isotype control antibody, and 0.1mg anti-CD24 monoclonal antibody. Treatments were initiated one week post tumor cell inoculation (to allow establishment of micrometastatic disease) and were repeated every fourth day, for a total of four treatments. Assessment using luminescent imaging of the lungs revealed a decreased tumor burden for mice treated with anti-CD24 antibody (mean luminescence of 4.8×10^5 versus 1.3×10^6 for IgG1 isotype mice). Furthermore, treatment resulted in increased overall and disease specific survival, approaching significance in the small cohorts evaluated ($P=0.076$). Together, these findings suggest CD24 merits further investigation as a target for anti-metastatic therapeutics in bladder cancer.

Chromatin State Mapping of a Cancer Stem Cell in Neuroblastoma

Shaili Shah - Biology

Histone modifications correlate with gene transcription. However, how epigenetics controls cell fate is not clearly defined. While some histone modifications correlate with gene activity, such as Histone H3 lysine 4 trimethylation (H3K4me3), others, such as lysine 27 trimethylation (H3K27me3) are associated with gene silencing. To better understand the role of epigenetics in differentiation and cell fate, these two modifications were profiled using chromatin state mapping in neuroblastoma cells. Neuroblastoma is a heterogeneous solid tumor composed mainly of three cell types: I, S and N cells. The highly malignant I-type cancer stem cell differentiates to both benign S-type and less malignant N-type cells. Regions of the neuroblastoma genome are modified with either H3K27me3 or H3K4me3, and comodification occurs on a small subset of regions. H3K4me3 correlates with gene expression and DAVID analysis shows different combinations of histone modifications are associated with genes that have distinct functions. These three cell types also show differential mRNA expression of the proteins responsible for the methylation status of H3K27. While the histone methyltransferase KMT6 shows decreased expression in the differentiated cell types, the expression of the two demethylases, KDM6A and KDM6B, have a more variable expression profile. Further experiments aimed at uncovering how cancer stem cell fate is correlated with H3K27 methylation are underway.

Aridity and Fog Deposition as Controls on C3/CAM Photosynthesis and N-cycling in *Welwitschia mirabilis*

Keir Soderberg – Environmental Science

Environmental controls on photosynthesis in *Welwitschia mirabilis* are evaluated through $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses of leaf material from 26 individuals in the southernmost population of this Namib Desert endemic. The coastal Namib Desert in southwestern Africa is hyperarid in terms of rainfall, but receives up to 100 days of fog each year. This climate regime leads to interesting water relations among the desert flora and fauna. Among many enigmatic characteristics, photosynthesis in *W. mirabilis* has puzzled researchers since the 1970's. Although it is predominantly a C3 plant, $\delta^{13}\text{C}$ ranges from -17.5 to -23.5‰ in natural habitats, and can be as enriched as -14.4‰ under artificial growing conditions. Recently the CAM pathway has been confirmed, but the driver for CAM utilization has not been identified. In this study we incorporate new $\delta^{13}\text{C}$ compositions for plants in the middle of the 100 km aridity gradient which spans the natural distribution of *W. mirabilis*. Initial results show an enriched $\delta^{13}\text{C}$ signal (-20‰) in the more exposed individuals compared with those in a sandy drainage depression (-22‰). In addition, the documented correlation between rainfall and $\delta^{15}\text{N}$ found in Kalahari C3 plants is used to interpret the $\delta^{15}\text{N}$ values in this *W. mirabilis* population. Initial results indicate that the fog deposition may significantly affect the nutrition of these unusual plants from the Namib Desert.

Peripubertal development of sex differences in androgen receptor associated membranes and fibers

Lucia Tejada - Neuroscience

Puberty is considered by some to be a second critical period when gonadal hormones may organize differences in adult brain and behaviors. The medial preoptic area (mPOA), contains several well studied sexually dimorphisms. For example, a sexually dimorphic region has been identified with Nissl staining and with calbindin immunoreactivity, and within the anteroventral periventricular nucleus tyrosine hydroxylase immunoreactivity has revealed another sexual dimorphism. The number of cells containing androgen receptor (AR) protein or mRNA is also sexually dimorphic in adults. To assess the peripubertal contributions to AR sexual dimorphisms in this region, we used transgenic mice (C57BL/6J) bearing a modified AR gene. In these mice, cells that express AR also express two reporter molecules, LacZ for nuclear visualization and placental alkaline phosphatase (PLAP), which stains cell membranes and associated fibers. One advantage of this method is that the activity of the PLAP is not affected by steroid hormones thus sex differences are not a reflection of differences in circulating testosterone levels.

We collected brains from male and female mice before, during, and after puberty (PN21, 35 and 50). We quantified the expression of AR in two sexually dimorphic regions, the mPOA and the bed nucleus of the stria terminalis (BNST). The peripubertal period does not produce a sexual dimorphic expression of AR positive cells and membranes in neither the BNST or mPOA. During puberty the average intensity of AR positive membranes ($p < 0.001$) and number of cells ($p < 0.001$) decreases in the mPOA. The BNST also shows lower values of average intensity in AR positive membranes ($p < 0.001$) and number of cells ($p < 0.001$) during puberty. This work was supported by NIH grants RO1 MH057759 and T32 HD007323.

"Deletion of adenosine A2A receptors on CD8 T cells enhances their accumulation in tumors and tumor-draining lymph nodes, as well as their ability to control tumor outgrowth"

Isaah Vincet - Microbiology

Previous work has demonstrated that ablation of adenosine A2A receptor (A2AR) expression in tumor-bearing mice enhances tumor control. However, it is not yet clear what cells and functions are influenced by A2AR signaling. Here we show that the ability of adoptively transferred OT-I CD8 T cells to control ovalbumin expressing B16 melanomas in A2AR^{+/+} recipients is enhanced if the A2AR gene is ablated in the OT-I cells. This demonstrates directly that the ability of CD8 T cells to control tumors is influenced by adenosine. To further understand the functions of CD8 T cells influenced by A2AR deletion, we examined the activation, proliferation, and survival of A2AR^{+/+} and A2AR^{-/-} OT-I cells in tumors and tumor-draining lymph nodes (TDLN) after co-transfer. While activation and proliferation of both populations in TDLN was comparable, the accumulation of A2AR^{-/-} cells in both TDLN and tumor was enhanced relative to A2AR^{+/+} cells. Preliminary data suggest that this is due to enhanced survival of A2AR^{-/-} CD8 T cells in TDLN. This enhanced accumulation does not occur in the lymph nodes of mice immunized with bone marrow derived dendritic cells or Vaccinia virus, suggesting that it reflects alterations in the level of adenosine specifically in TDLN. We theorize that enhanced accumulation of A2AR^{-/-} CD8 T cells is at least partially responsible for their increased ability to control tumors.

The A2B Adenosine Receptor Stimulates Dendritic Cell IL-6 and Promotes De Novo Generation of Th17 Cells

Jeff Wilson - Medicine

The development of appropriate effector CD4⁺ T cell subsets is critical for host immune defense against specific pathogens. Until recently effector CD4⁺ T cells were categorized into two subsets, with Th1 cells specialized for intracellular cell-mediated immunity and Th2 cells evolved to generate antibody responses and defend against extracellular pathogens. In the past few years additional subsets with unique phenotypes and functions have been recognized and include so-called Th17 and Treg cells. Thus, an important goal in immunology is to elaborate factors, both pathogen and host, that control the differentiation of naïve CD4⁺ T lymphocytes into effector subsets. Adenosine, an endogenous purine nucleoside, is up-regulated during inflammation and is known to exert a myriad of activities on many immune cells. To address a role for adenosine in shaping the development of CD4⁺ T cell responses we set up an in vitro model of T cell differentiation that consisted of naïve CD4⁺ T cells in co-culture with dendritic cells (DC), potent antigen-presenting cells that are instrumental for initiating T cell responses. Using this model we assessed the role that a stable non-selective adenosine analogue had on CD4⁺ T cell differentiation.

Here we report that adenosine promotes Th17 responses by acting on DC adenosine A2B receptor (A2BAR). Naïve CD4⁺ T cells co-cultured with DC in the presence of a non-selective adenosine analogue results in the differentiation of cells that express elevated levels of signature Th17 molecules such IL-17, IL-22 and $\text{ror-}\gamma\text{T}$. Specific experiments suggest that: 1) the effect is mediated through DC, and not due to effects on T cells; 2) the A2BAR is largely responsible and; 3) the effect is associated with specific mediators. For example, the induction of Th17 cells is dependent on the presence of IL-6 and TGF- β and the absence of IL-12 – conditions which are met in the NECA treated DC. Furthermore, co-cultures conducted in the presence of TGF- β 1 induce adaptive FoxP3⁺ Treg but this is blocked by NECA and Th17 cells emerge. Although previously implicated in Treg development and function, these data suggest that under some circumstances, adenosine acts via A2BAR expressed by DC to favor the Th17 lineage.

Transport Defective Mutants: A TonB-Dependent System

Cheng Yen - Chemistry

Gram-negative bacteria are responsible for a wide range of potentially fatal diseases such as meningitis and pertussis. Gram-negative bacteria such as *Escherichia coli* utilize TonB-dependent transporters (TBDTs) to gather limited, essential nutrients such as iron and vitamin B12 (cyanocobalamin) from the surrounding environment, which are too large to passively diffuse through porins in the outer membrane. These transporters are termed TonB-dependent because they presumably couple to the transperiplasmic protein TonB, which hypothetically delivers the energy required for transport. Despite the existence of several high-resolution crystal structures of these transporters, the molecular mechanism of TonB-dependent transport remains an enigma.

BtuB is a well-characterized transporter for vitamin B12 and is distinguished by its unique hatch and barrel regions. In addition, it possesses a conserved coupling motif known as the Ton box, which seems to function in coupling to TonB. The Ton box of BtuB remains folded within the hatch region when no substrate is present, but continuous wave electron paramagnetic resonance (CW EPR) experiments have shown that the addition of substrate causes the Ton box to become disordered and thereby solvent accessible, indicating that the substrate-dependent unfolding of the Ton box could possibly be a key transmembrane signaling mechanism for TonB interaction. Past research has demonstrated the existence of mutants that demonstrate atypical Ton box behavior whereby the Ton box that is unfolded regardless of whether or not substrate is present. More importantly, the mutants are unable to transport B12, rendering them transport defective.

The Ton box mutation could have possibly interfered with the transport mechanism by disrupting the interaction between the Ton box and BtuB or by inducing a structural change in the transporter that renders it transport defective. EPR spectroscopy was utilized to assess the binding affinities of the transport defective mutants to determine if there are significant differences in binding affinity when compared to normal, wild-type samples. X-ray crystallography will be used to determine if the Ton box mutation induces any structural changes in the hatch and barrel regions of BtuB. By studying why these transport defective mutants are unable to transport B12, a better understanding of the mechanism behind TonB-dependent transport will be achieved.

Asymmetric Activation of the Amygdala Following Emotionally Arousing Situations

Erica Young - Psychology/Neuroscience

Findings collected from functional imaging studies reveal that presentation of pleasant or unpleasant emotional stimuli increases activity within the human amygdala. New evidence suggests that this activation may be relatively selective since the valence of environmental stimuli may dictate whether activation is greater in the amygdala of one hemisphere relative to the contralateral side. This view is supported by data showing that in laboratory animals, learning and successful memory of responses acquired with aversive or unpleasant stimuli requires an intact right amygdala more than the left. These types of findings suggest a valence-dependent recruitment of the amygdala such that activity in the right amygdala is initiated by exposure to negative experiences while more pleasant or rewarding events elicit greater responses in the left amygdala. However, few studies have been conducted to unravel the mechanisms underlying the selective, valence-dependent initiation of activity in the amygdala of opposing hemispheres. The neurotransmitter norepinephrine is an important regulator of stress, arousal, and limbic brain activity essential for encoding new emotional events into memory storage. As such, the asymmetric pattern of amygdala activity observed in response to pleasant vs. unpleasant learning conditions may be initiated by the selective release of norepinephrine within this structure.

To address this possibility, the present study determined whether exposure to positive or negative learning conditions differentially initiates fluctuations in norepinephrine release between the left and right amygdala respectively. Separate groups of laboratory animals were trained over a 10 day period to press an extended lever on a fixed ratio schedule (5 presses each trial) to obtain either a large (10 sucrose pellets; 450mg) or small reward (only 1 sucrose pellet; 45mg). On Day 11, the experimenters violated the subject's expectations of reward outcome to create a positive or negative learning experience. On this day, half of the subjects in the small reward group received 10, rather than the expected 1 food pellet after completing each series of bar presses. Similarly, half of the subjects assigned to the large reward group were given only 1 as opposed to the normal 10 pellet after completing the regimen of bar presses. The consequences of violating the magnitude of expected food rewards on arousal as reflected in norepinephrine release in the amygdala was assessed prior to and up to two hours following this experimental manipulation.

Findings indicated that animals shifted from the high to low food reward (negative arousal) had significantly higher levels of extracellular norepinephrine in the right basolateral amygdala compared to the left. Conversely, animals that were shifted from the low food reward group to the high (positive arousal) showed a significant increase in norepinephrine release in the left basolateral amygdala relative to the right. These results indicate that during negatively arousing learning tasks there is greater engagement of modulatory mechanisms in the right amygdala whereas positively arousing learning tasks result in greater left amygdala involvement. Using *in vivo* microdialysis to show that there is valence dependent asymmetric release of norepinephrine in the BLA extends findings from functional scan studies and is the first step in understanding how asymmetric activation of limbic structures contributes to emotional memory formation.