The Effect of Endocannabinoids on Carbachol Induced Contractions in the Rat Uterus
2008 REU Animal Behavior Abstract

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Interest in endogenous cannabinoids has been generated by the well-document analgesic properties of exogenous cannabinoids, most familiarly Δ^9-tetrahydrocannabinol. The endocannabinoid pathway is a complex signaling system involving CB1 and CB2 G-protein coupled receptors, which are activated by lipid ligands. Previous research has delineated the roles of both receptors in analgesia and nociception by using knockout mice and genetic studies in combination with pain model tests, but - due to pain tolerance differences in the strains of rat used and variation in pain models - previous tests on the properties of the CB1 and CB2 receptors have been conflicting. Regardless, regulation of the endocannabinoid pathway has the potential to regulate pain response and treat pain disorders. Part of the established endocannabinoid system involves a calcium-dependent transacylase-catalyzed enzymatic phosphorylation and hydrolysis that produces of N-acylethanolamines. Endocannabinoids such as anandamide have been shown to antagonize the spontaneous contractility of muscarinic smooth muscle ileum tissue, repressing the observed amplitude of contractions in a concentration-dependent fashion. Contractions in uterine and other smooth muscle tissue are stimulated by the parasympathetic nervous system's release of acetylcholine, and thus an organ bath experiment was used to manipulate the pathway and observe resulting contractions. Tissue harvested from rats determined to be in estrus of a regularly proceeding cycle was dissected into four samples and mounted in buffer solution at 32 degrees Celsius. Through a calcium channel blockade, indomethacin inhibits prostaglandin production and thus suppresses smooth muscle contractions. By incubating the uterine tissue with indomethacin, the spontaneous contractions characteristic of estrus were suppressed and any subsequent contractions were assuredly pharmacologically induced by carbachol, a nonselective and cholinesterase-resistant muscarinic receptor agonist. As a potent CB1 receptor agonist, methanandamide was introduced to the system prior to carbachol additions to investigate the role of endocannabinoids on smooth muscle tissue contractions. Preliminary results support methanandamide's ability to reduce or inhibit contractions in uterine tissue when compared to a DMSO control sample. Because the ability of a pharmacological agent to inhibit uterine contractions has potential as a candidate drug for women suffering from dysmenorrhea and other sexual pain disorders, future research should continue.

Sexually Dimorphic Communication Behaviors in *Sternarchogiton nattereri*

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The electrocommunication signals of electric fish differ across individuals, species, and sexes. These signals are produced by an electric organ (EO) in the fishes' tail. In species that produce 'wave-type' discharges, including fish in the family Apteronotidae, the EO discharge (EOD) consists of highly rhythmic, quasi-sinusoidal voltage changes around the fishes' body. Each individual emits a particular electric organ discharge frequency (EODf), but in social situations individuals show variation in EOD modulations, known as chirps and gradual frequency rises (GFRs). The ease with which electrocommunication signals are observed and quantified, as well as the variation in EOD signals, and the sexual dimorphism in these signals, make weakly electric fish ideally suited for studying the evolution of sexual dimorphism in communication behavior. Although, EOD waveform, EOD frequency and EOD modulations have been studied in many apteronotid species (Turner et al. 2007), few studies have explored sex differences in chirping and EOD behavior. Of the apteronotid species studied, there is variation in EOD signals and in the degree of sexual dimorphism for those signals. For example, *Apteronotus leptorhynchus* and *Apteronotus albifrons* are sexually dimorphic for EODf, with *A. leptorhynchus* males producing higher EODf and *A. albifrons* males producing lower EODf relative to females (Zakon and Dunlap 1999). Conversely, *Adontosternarchus devenanzii* is sexually monomorphic for EODf, but males differed in chirp structure when compared to females (Zhou and Smith 2006). The purpose of this study is to (1) look at sex differences in a little studied apteronotid species called *Sternarchogiton nattereri* and (2) obtain sequence data from the COI gene of *S. nattereri* for an apteronotid phylogeny that will allow us to look at the evolution of communication behavior within this family. We found that like *A. devenanzii*, *S. nattereri* is sexually monomorphic for EODf ($p = .85$), chirp duration ($p = .25$) and chirp rate ($p = .62$). However, *S. nattereri* males produce chirps with a greater amount of frequency modulation as compared to females ($p = .013$). Preliminary sequence data puts *Sternarchogiton nattereri* within the Apteronotidae, and indicates that *S. nattereri* is more closely related to *Sternarchorhynchus* than to *Apteronotus*. These data expand on existing evidence for the lability of communication behaviors in Apteronotidae. Together, our behavioral and phylogenetic data is well suited for studying sexual dimorphism in communication behaviors, and provides insight into the strength of natural and sexual selection that shapes these traits.

Variation of signal detection in *Sceloporus undulatus* lizards

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Research in animal communication suggests that visual signal properties often vary greatly between and within individuals in a variety of social contexts. For example, in many species of lizards, males produce different complex motion displays used in territorial and courtship contexts. While it is widely known that visual signals emitted by senders can exhibit great variation in efficacy and design, far less is understood whether and how receivers vary in the ability to detect and respond to different complex signals. In this study, we tested for variation of signal detection within and between receivers. Specifically, we tested whether lizards (*Sceloporus undulatus*) exhibit differential signal detection between the left and right visual fields, a phenomenon known as visual laterality. Additionally, we tested whether male and female *S. undulatus* lizards differ in their ability to detect two types of motion signals displayed by males: courtship and territorial. We assayed visual performance from each visual field and each sex by measuring the latency to detect the two male display types presented by a robotic lizard model. Our results show that the left and right visual fields of both males and females differ in the ability to detect both display types. Specifically, males were overall better at detection using their right eye and were also better than females at detecting courtship displays. These results demonstrate that while signal efficacy can greatly depend on the receiver's ability to detect and respond to a sender's signals, variation both within and between different receivers can affect the direction of signal evolution. These findings suggest that sexual asymmetry and laterality of visual ability may play an important role in the evolution of visual communication systems and should be taken into consideration when studying signal design and efficacy.

Sexual dimorphism of electrocommunication signals across populations of the weakly electric fish *Apteronotus albifrons*.

REU Abstract

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Electrocommunication signals in electric fish vary widely within and among species. The genus *Apteronotus* produces electrical signals called electric organ discharges (EODs) that function in both electrolocation and communication. The sinusoidal EOD can be characterized by a particular frequency, the EOD frequency (EODf). The EODf, as well as modulations of the EOD called chirps and gradual frequency rises (GFRs), can be specific to species, sex, or individual. We examined diversity in sexually dimorphic communication signals from populations of *Apteronotus albifrons*, the black ghost knifefish. Recent data suggests that black ghosts exhibit a range of intraspecific variation (Ho and Smith, unpublished data, 2007). In this study, we described the sexually dimorphic electrocommunication behaviors of two previously unstudied populations of black ghosts, and obtained data on their phylogeographic relationships to examine how behavioral diversity has evolved. Twelve individuals from the Orinoco drainage in Columbia (CO-oct) and five individuals from the Amazon drainage in Peru (PE) were recorded individually, using playback stimuli to elicit chirps and GFRs. Additionally, we sequenced the mtDNA cytochrome-b region in these individuals in order to understand how populations of black ghosts from across South America are related. We compared our data with a previous study examining populations from the Amazon drainage in Brazil (BR) and Columbia (CO-hf) and from the Orinoco drainage in Columbia (CO-jun). We found that the CO-oct individuals were sexually dimorphic for EODf and chirp structure. This EODf data is consistent with data from CO-jun individuals. CO-oct females, however, produce chirps with greater amounts of frequency modulation than do males, a condition that is reversed in the CO-jun population, where male FM is greater than that for females. PE individuals were sexually monomorphic in EODf, which corresponds with prior findings for the BR individuals. After sequencing, we found that the CO-oct and PE populations are genetically distinct from each other, with cytochrome-b sequences differing by 3%. In addition, we found that CO-jun and CO-oct populations showed no genetic differentiation, while haplotypes from the PE individuals were most closely related to BR fish. Populations from the same river systems are therefore more genetically similar than populations in separate river systems. This is consistent with the hypothesis that diversity in sexually dimorphic EOD behavior has evolved in allopatry, with South American watersheds acting as barriers for reproductive isolation.

Nicotine Entrainment Treated with Mecamylamine Decreased Post-injection Activity

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Daily nicotine administration in rats has been shown to act as a zeitgeber for the entrainment of circadian activity rhythms. Nicotine-induced entrainment shows both anticipatory activity beginning 2 hrs before the injection time and a drug-evoked increase in post-injection activity. A treatment for nicotine abuse may lie in the ability to eradicate entrainment to the drug administration time. Mecamylamine, a nicotinic acetylcholine receptor antagonist, has been therapeutically used to help decrease nicotine consumption. The present study used 16 adult female Sprague-Dawley rats (*Rattus norvegicus*) divided equally into 2 groups: one dosed with nicotine (NIC) and the other with saline (SAL). The subjects were housed in wheel boxes under constant dim light and rate limited feeding. The rats underwent two subcutaneous injection series (1 mg/kg nicotine or 1 mL/kg saline, corresponding to group membership), followed by 2 mecamylamine (0.57 mg/kg) treatment days, and 4 baseline days were administered over 36 days. Mecamylamine treatment showed significant decreases in post-injection activity during the treatment days, but pre-injection withdrawal activity increased compared to the nicotine injection series. The partial, but not complete, elimination of entrained behavior provides evidence that separate oscillators modulate drug seeking and drug-evoked behaviors.

Regulatory Competence in Mice

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Understanding behavior requires knowledge of the stimuli that initiate and direct an organism's actions. Taxes are simple, stimulus-bound responses that are seen as elements of more complex behavior. The present study investigated thermotaxis in (4-5 days of age) infant mice (*Mus musculus)* Thermotaxis implies movement towards a heat source. The present series of experiments was designed to determine whether infant mice are thermotaxic. Pups were placed individually on the 90cm-long thermocline and observed for two hours. The surface temperature gradient ranged from 22.9°- 33.5° C and pups were placed in the center 27.7° C. The individual pups stopped on average at 32.8°C. The second steeper gradient ranged from 24.4°- 39.4° C and pups were started at 31.6° C. The individual pups stopped on average at 33.3°C. To determine whether pups could regulate to a cooler region, some were placed at the warmest (39.2° C) end of the second gradient. Pups placed in a hot environment did not move to a cooler region. 4-pup groups were then compared to individual pups according to rate of movement along the original gradient. As a group of four, the pups exhibited faster detection of the gradient and moved to the warm ends of the gradient more rapidly than individuals. On average, the group stopped at 32° C on the gradient. These findings indicate that pups are limited to the ability to thermoregulate when seeking warmth but are unable to adjust to cooler temperatures. The pups detected a temperature gradient and through irregular motions gradually moved to the warm regions of the gradient. The function of the group suggests sensitivity beyond the capabilities of the individual infant mouse. It was concluded that infant mice are capable of limited behavioral thermoregulation. This rejects formal definition of taxis as an uncontrolled or mechanized reaction, which does not permit the option of regulation.

The role of social feedback in gesture production of 14-18 month-old infants and their caregivers

2008 REU Animal Behavior Abstract

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Examining the origins of communicative development is necessary to understand how individuals learn to effectively communicate with social partners. Previous research revealed how caregiver social feedback, sensitive or redirective patterns of behavior, can modify both cognitive and vocal development. Gesture research has suggested that producing gestures forecasts sophisticated vocalizations; research has revealed a link in the lateralization between gestures and vocalizations. The purpose of my study was twofold. First, I wanted to examine whether infant gesture production can be influenced by their caregiver's feedback. Second I wanted to examine if caregiver gesture production was related to infant gesture production. Over two visits we systematically manipulated caregiver social feedback with sensitive and redirective patterns of behavior to examine how these patterns could influence gesture production. Results show there was a significant decrease in the overall gesture production of infants in the redirective condition, however, there were no differences of gesture production in the sensitive condition. Furthermore, there were no differences in caregiver gesture between the two conditions. In addition, we found that more developmentally advanced vocalizations were produced in the redirective condition. This study demonstrates that infant gesture production can be shaped by caregiver social feedback and there may be a potential dissociation between infant vocal development and gesture production.

The Effect of the Environment and Shoal Composition on the Behavior of Zebrafish (*Danio rerio*)

2008 REU Animal Behavior Abstract

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The knowledge of zebrafish behavior extends to the usefulness of aggression (Basquill *et al*, 1988), the genetic variability of boldness (Wright *et al*, 2003), and the introduction of shoaling preferences (Engeszer *et al*, 2007). This experiment measures the effect of various tank environments on three different wild strain populations as well as the effect induced by the introduction of lab strain fish into a shoal of zebrafish from a wild population. Is there a combined effect between the tank environment and the shoal composition on the zebrafish's behavior? Does the tank environment affect the behavior? And finally, does the shoal composition influence the behavior of the zebrafish? To assess the tank and shoal effect on the zebrafish, we designed four tanks that contained a combination of flow and vegetation with all combinations present. The wild strain fish were placed either in control groups or mixed groups that contained two individuals from the lab strain of zebrafish. The groups experienced four behavioral tests that included the latency to feed, the total number of chases, the maximum shoaling distance, and the boldness of the fish one minute after the entrance of a predator. Shoaling distance was significantly affected by flow in all of the populations tested. While no other behavior displayed a significant effect for all three populations, the tank environment was found to be significant for certain behaviors within populations. The addition of the lab strain zebrafish into the shoal composition affected the boldness of each wild strain population. We also found that for the wild strain Panigram all of the behaviors tested were affected by the presence of the lab strain zebrafish.

Functional Subdivisions of the Medial Amygdala in Male Zebra Finches (*Taeniopygia guttata*)

2008 Animal Behavior REU Abstract

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Across all land vertebrates, the medial amygdala plays an integral role in the neural network associated with social behaviors and is essential in determining whether an animal will respond to social stimuli in an aggressive, affiliative, or defensive manner. While the gross architecture of the social behavior network is common to all vertebrates, there has been little investigation into the functional subdivisions of the MeA in non-mammals, and it is not yet clear whether this subnuclear organization is similarly conserved. It has been shown that the rodent MeA consists of three subnuclei that are differentially involved in various aspects of social behavior—the anterior MeA, the posterior ventral MeA, and the posterior dorsal MeA (Choi et al, 2005). The present experiments were designed to determine whether the avian MeA exhibits functional subdivisions that are homologous to those in mammals and to expand the range of experimental social stimuli beyond those typical of rodent studies. To this end, male zebra finches (*Taeniopygia guttata*) were exposed to one of five behavioral conditions: control, presence of a heterospecific male, presence of a conspecific male, mate competition, or copulation. The subjects were intracardially perfused, and their brains sectioned. The brains were then immunocytochemically labeled for Fos protein, a marker of neuronal activation, and the relative densities of neuronal activation in the three subdivisions of the MeA were analyzed. Differential patterns of activation among experimental groups will help to elucidate the functional subdivisions of the avian MeA.