The highlight for May is by Dr. G. Andrew Mickley from Baldwin-Wallace College. As described in the highlight, Dr. Mickley's interest in and work with taste aversion learning has changed dramatically over the almost 4 decades in which he has been an active researcher in the field. Working with Phil and Mike Best, Dr. Mickley began his career in much the same way that many of us did in the early years of the field, i.e., exploring the limits of the phenomenon of aversion learning and the conditions under which it might or might not occur. His initial work assessed aversion conditioning with exteroceptive stimuli and whether such stimuli might support conditioning themselves (as a secondary or conditioned reinforcer). Dr. Mickley found his interests over time turning from questions regarding the specific conditions of aversion learning to its utility as a tool to explore a host of other interesting and important phenomena, in his case, the physiology and pharmacology of learning and memory. While trying to assess the feasibility of memory (CTA) transfer with fetal transplants, he and his colleagues reported an atypical finding of memory enhancement with the NMDA channel blocker, ketamine (a compound that generally impairs learning and memory). His follow-up work with this finding revealed it to be specific to a window of fetal development (around E18). Extending this work to other preparations, e.g., taste reactivity and positive contrast, confirmed these memory enhancing effects of ketamine at this stage in fetal development. The temporal window of these behavioral effects in both aversion and non-aversion learning was supported by his work on parallel ketamine-induced changes (enhancements) of NMDA NR2B glutamate receptors. Subsequent to these investigations on drug-induced changes in learning and memory, Dr. Mickley used immunohistochemical techniques to assess changes in protein expression in various brain areas. In this work, he and his colleagues demonstrated that brain activity associated with spontaneous recovery of extinguished taste aversions was not similar to that of a naïve animal, suggesting that extinction of taste aversions was not simply an unlearning (but more likely the acquisition of a new response). Dr. Mickley's summary not only highlights his own history, but that of the field in general, as the focus on aversion learning has shifted from the analysis of a unique form of learning to a preparation with clear neuroscience and clinical applications.

Learning about learning via the CTA paradigm

G. Andrew Mickley, Ph.D.
Department of Psychology
Neuroscience Program
Baldwin-Wallace College
amickley@bw.edu

I can trace my interest in CTA back to my graduate school days and my initial studies at the University of Virginia as Phil Best, Mike Best and I worked on a variety of topics relevant to learning and memory. In those early days we were especially interested in testing the sensitivity

of the CTA paradigm and pushing it to its limits. Could rats go beyond associating two interoceptive stimuli (taste and illness) and also learn to associate visual or other exteroceptive stimuli with LiCl-induced malaise? Could a secondary reinforcer be used as a US? What we discovered was that, with some specialized training, rats could associate a distinctive context with illness. Moreover, that space, when later associated with a taste, could be used as an effective US (Best, Best & Mickley, 1973). The CTA paradigm was very robust indeed, and it would be one that captured my interest over a long and enjoyable career in science.

Of course, the 1970s were a time of turmoil and war in Vietnam. I found myself yanked out of graduate school and into the U.S. Air Force which, unexpectedly, I enjoyed. As an Air Force scientist, I continued to work in behavioral neuroscience in some excellent National labs. But only at the end of my 21-year Air Force career was I able to return to studies that involved CTA.

I had been doing some experiments evaluating the efficacy of neural transplants to attenuate or reverse behavioral effects resulting from damage to the hypothalamus or the hippocampus (Mickley, Teitelbaum & Reier, 1987; Mickley, Ferguson, Nemeth, & Mulvihill, 1990; Mickley, Ferguson, Mulvihill, & Nemeth, 1991). I later received a small grant to do a high-risk study aimed at determining if neural grafts might also be able to carry with them memories from one animal to another. Since the neural transplant work of the day used still-mitotic fetal brain tissue as the graft, I needed to find something that fetuses could learn. Most of the sensory systems are not well developed in the fetus, but I became aware of some elegant studies done by Bill Smotherman and Scott Robinson (1985; 1990; 1991) who were able to demonstrate that rat fetuses could learn a CTA as early as E17. Moreover, the neural substrate of CTA learning had been partially described and, therefore, I knew that the candidate location for the CTA "engram" (if there was a single one) was to be found along the taste pathway running from the nucleus of the solitary tract (NTS), parabrachial nucleus (PBN) and then to the basolateral amygdala (BLA) and gustatory neocortex (GNC) (Houpt et al., 1994; Yamamoto & Fujimoto, 1991; Yamamoto, Shimura, Sako, Yasoshima & Sakai, 1994). Equipped with this information, I began a series of what, in retrospect, seem like foolhardy studies to determine the efficacy of using neural transplants to transfer a memory from one animal to another. Fetal rat brain tissue may be grafted into adult brains and thrive. If I taught a rat fetus a CTA to the taste of saccharin and then transplanted portions of their brains into adult rats, might the host rats spontaneously avoid saccharin?

There were a variety of technical challenges that needed to be overcome in order to do this study. One of the first requirements was to establish the optimal age at which fetal rats could acquire a robust CTA. Smotherman and Robinson had done most of the heavy lifting here (see Smotherman & Robinson, 1990; 1991), but there were some details that needed to be worked out to determine the best fetal age for our purposes. Second, I needed to balance this information with what I knew about rat brain development in order to maximize the probability that the conditioned brain nuclei of interest were still mitotic and, therefore, likely to survive the grafting process and to remain viable in the host brain.

In the course of this pilot work, I was anesthetizing pregnant dams before exteriorizing the uterus and injecting fetuses with saccharin (CS) and LiCl (US). Fetuses, so conditioned *in utero*, were then replaced into the dam, allowed to be born a few days later and then, eventually, the neonates

were tested to see if they had acquired/retained the CTA. For a period of time, we had run out of our usual anesthetic drug (sodium pentobarbital) and I had cavalierly experimented with other anesthetic combinations – some of which included the N-methyl-D-aspartate (NMDA) receptor blocking drug ketamine HCl. When my colleagues and I began to notice variability in the strength of CTAs of the neonates we were testing, we went searching for reasons why. Of course, the systemic anesthesia administered to the pregnant dam readily crossed the placental barrier. Was it affecting the strength of fetal CTA conditioning? As the literature now shows, of course it was (Mickley, Lovelace, Farrell, & Chang, 1995)!

At this same time, I was leaving the Air Force and moving to an academic appointment at Baldwin-Wallace College. Sadly, the original aims of the neural transplant study were caught in the shuffle. But my new Ohio laboratory, now consisting almost exclusively of undergraduate collaborators (Mickley, Kenmuir, & Remmers-Roeber, 2003), vigorously pursued a series of compelling questions about the role of glutamate receptors in fetal brain development and CTA. A colleague (Dawn Remmers-Roeber) and I visited Bill Smotherman's lab (SUNY, Binghamton) and developed some new methodologies of behavioral observation in fetuses. Our initial Ohio experiments surprised us by showing that the acquisition of a CTA by E18 rat fetuses was potentiated by a single maternal injection of ketamine (Mickley, Lovelace, Farrell, & Chang, 1995; Mickley, Remmers-Roeber, Dengler, Kenmuir, & Crouse, 2001). This finding was novel, since, up to this point, NMDA receptor blockade had been associated exclusively with learning and memory *impairments* in adult animals. Subsequent studies indicated that ketamine's enhancement of CTA acquisition was not detectable in P0 neonates where, instead, the more-typical drug-induced amnesia was found (Mickley, Schaldach, Snyder, Balogh, Len, Neimanis, Goulis, Hug, Sauchak, Remmers-Roeber, Carter, & Yamamoto, 1998).

Further studies on fetal rats of various ages employed not consummatory measures of taste aversion but rather oral-facial indicators of aversion (e.g., mouthing, licking and gaping responses) following oral lavage of the aversive saccharin. The methods of these studies in many ways paralleled those done by Grill and Norgren (1978) as they described the taste reactivity method of detecting aversive responding. By using these techniques, we were able to determine that CTA learning was potentiated by ketamine if it was administered before CS exposure on E18. But ketamine produced impairments in both CTA learning/retention and a non-associative taste recognition task if the drug was administered just 24 hours later on E19 (Mickley, Remmers-Roeber, Crouse, & Peluso, 2000a,b; Mickley, Remmers-Roeber, Dengler, Kenmuir, & Crouse, 2001). This ketamine paradox suggested that something very special and interesting might be happening in rat brain neuropharamacology and brain development between E18 and E19.

We eliminated alternative explanations for the ketamine paradox and attempted to determine its generalizability to other learning phenomena. For example, we used HPLC methods to match the levels of brain ketamine in our subjects of different ages that were receiving ketamine via either maternal circulation or direct (i.p.) injection (Mickley et al., 1998). The ketamine treatments did not seem to be changing the taste perception of rats nor their ability to experience the malaise produced by the LiCl (Mickley, Remmers-Roeber, Dengler, McMullen, Kenmuir, Girdler, Crouse, & Walker, 2002). We explored non-associative memory paradigms such as positive contrast (PC) and demonstrated that, in an age-dependant manner, fetal rats exhibited enhanced

mouthing and licking responses to a taste of 3% saccharin if it followed an initial previous exposure to 1.5% saccharin 24-hours earlier. This PC effect was not observed in control rats that received two sequential exposures of 3% saccharin (Mickley, Kenmuir, Dengler-Crish, McMullen, McConnell & Valentine, 2004). The PC effect emerged developmentally on E19, but if E18 rats were given ketamine before the first taste exposure it could be detected earlier – suggesting again that NMDA receptor blockade was somehow enhancing memory formation in these younger animals (Mickley, Biada, Kenmuir, Yocom, Wellman, Hoxha, & Hoxha, 2005). Further, we found that the surprising memory enhancing effect of ketamine administration on E18 apparently persisted in very subtle ways even into adulthood as revealed by water maze testing (Mickley, Kenmuir, McMullen, Snyder, McConnell, Likins-Fowler, Valentine, Weber, & Biada, 2004).

Most recently, we have gained some insights regarding putative neural mechanisms of the ketamine paradox. For example, we have employed Western blot analyses and detected ketamine-induced enhancements in the level of NMDA NR2B receptors if the drug was given on E18 but not if it was administered on E19 (Mickley, Biada, Kenmuir, Yocom, Wellman, Hoxha, & Hoxha, 2005). Thus, we are starting to get a handle on possible neural mechanisms that produce the ketamine paradox.

My students and I have also been interested in going beyond the study of CTA acquisition and looking also at the extinction and spontaneous recovery (SR) of this defensive reaction to a learned fear. These studies, done in adult rats, have used *c-fos* protein immunohistochemistry (as a marker of neural activity) to identify brain areas engaged during each stage of the conditioning, extinction and SR process.

Underlying some of the studies that have attempted to identify brain nuclei involved in learning and extinction is a theoretical debate about the extent to which extinction represents unlearning or new learning (Wagner & Rescorla, 1974). Because extinction erases the signs of fear, it is tempting to assume that it erases the original learning. However, as Pavlov (1927) described in his original studies on classical conditioning, allowing time to pass following extinction frequently evokes the re-emergence or spontaneous recovery of the CR. The phenomenon of SR indicates that, even after many extinction trials, an animal retains a memory of conditioning that can provide a powerful basis for relapse. Instead of erasing the original learning, extinction gives the CS a second, and therefore ambiguous, meaning. In the case of SR, temporal cues may play a role in evoking the originally learned response (Bouton & Swartzentruber, 1991). Thus, the study of SR may have important clinical relevance. For example, fear sometimes spontaneously recovers between sessions of exposure therapy (Rachman & Lopatke, 1988).

Briefly, we have looked at the regional patterns of brain c-Fos protein expression following the acquisition, extinction and SR of a CTA in rats. We followed the time course of extinction until rats reached asymptotic levels of CS reacceptance. The experiments have generally supported the contention that CTA extinction is new learning rather than unlearning. For example, extinction of a CTA is accompanied by a burst of c-Fos expression in the gustatory neocortex (GNC) and medial pre-frontal cortex (mPFC). This pattern of neural activity is not seen in naïve rats before they acquired a CTA, suggesting that, following extinction, the brain is not simply returning to a pre-conditioned state (Mickley, Kenmuir, McMullen, Yocom, Valentine, Dengler-Crish, Weber,

Wellman, & Remmers-Roeber, 2004; Mickley, Kenmuir, Yocom, Wellman, & Biada, 2005). We have also observed enhanced activity in the basolateral amygdala (BLA) in the middle stages of extinction (dynamic phase; see Nolan et al., 1997) but not when the extinction memory has been firmly fixed (i.e., asymptotic extinction). SR of a CTA is accompanied by, for example, reversal of the burst of c-Fos expression in the GNC that is normally seen when rats extinguished the CTA (Mickley, Hoxha, Bacik, Kenmuir, Wellman, Biada,& DiSorbo, 2007).

Recently, we have noted that CTA extinction may be obtained by merely exposing the conditioned rat to the non-reinforced CS or, alternatively, exposing the rat to the CS explicitly unpaired with the US (EU procedure; Rauhut, Thomas, & Ayres, 2001). Both methods produce equal re-acceptance of the once-avoided saccharin. However, rats that undergo the EU procedure do not spontaneously recover their CTA, while the rats that undergo the CS-only exposures do show SR (data not yet published). Our current studies in this topical area are aimed at finding behavioral means to suppress SR of learned fears and discovering the patterns of brain activity that support extinction by these two different methods.

As I reflect on the 37 years I have been engaged in CTA research, it's clear that my interests in the paradigm have changed since those early days at the University of Virginia. At the start, most of us in the field were exploring the limits of the paradigm and describing how different methods and parameters could influence this unusual form of learning. But ultimately, for me, CTA has become an incredibly useful tool to help me and my students understand brain development and some of the basic neural mechanisms of learning, extinction and SR. The characteristics of CTA that make it special (e.g., one-trial learning, robust conditioning even in fetal rats, long retention and extinction times, etc.) continue to make it the method of choice in my laboratory.

References

Best, P.J., Best, M.R., & Mickley, G.A. (1973). Conditioned aversion to visual environmental stimuli resulting from gastrointestinal distress. *Journal of Comparative and Physiological Psychology*, *85*, 250-257.

Bouton, M.E., & Swartzentruber, D. (1991). Sources of relapse after extinction in Pavlovian and instrumental learning. *Clinical Psychology Review*, *11*, 123-140.

Grill, H.J., & Norgren, R. (1978). The taste reactivity test I: Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research, 143*, 263-279.

Houpt, T.A., Philopena, J.M., Wessel, T.C., Joh, T.H., & Smith, G.P. (1994). Increased c-Fos expression in nucleus of the solitary tract correlated with conditioned taste aversion to sucrose in rats. *Neuroscience Letters*, *172*, 1-5.

Mickley, G.A., Teitelbaum H., & Reier, P.J. (1987). Fetal hypothalamic brain grafts reduce the obesity produced by ventromedial hypothalamic lesions. *Brain Research*, *424*, 238-248.

Mickley, G.A., Ferguson, J.L., Nemeth, T.J., & Mulvihill, M.A. (1990). Neural grafts reverse behavioral deficits associated with early radiation-induced brain damage. *Brain Research*, *509*, 280-292.

Mickley, G.A., Ferguson, J.L., Mulvihill, M.A., & Nemeth, T.J. (1991). Early neural grafts transiently reduce the behavioral effects of radiation-induced fascia dentate granule cell hypoplasia. *Brain Research*, *550*, 24-34.

Mickley, G.A., Lovelace, J.D., Farrell, S.T., & Chang, K.S. (1995). The intensity of fetal taste aversion is modulated by the anesthesia used during conditioning. *Developmental Brain Research*, *85*, 119-127.

Mickley, G.A., Schaldach, M.A., Snyder, K.J., Balogh, S.A., Len, T., Neimanis, K., Goulis, et al. (1998). Ketamine blocks a conditioned taste aversion (CTA) in neonatal rats. *Physiology and Behavior*, *64*, 381-390.

Mickley, G.A., Remmers-Roeber, D., Crouse, C., & Peluso, R. (2000a). Ketamine blocks a taste-mediated conditioned motor response in perinatal rats. *Pharmacology, Biochemistry and Behavior*, *66*, 547-552.

Mickley, G.A., Remmers-Roeber, D., Crouse, C., & Peluso, R. (2000b). Ketamine blocks a taste recognition memory in fetal rats. *Pharmacology, Biochemistry and Behavior*, *67*, 575-581.

Mickley, G.A., Remmers-Roeber, D.R., Dengler, C.M., Kenmuir, C.L., & Crouse, C. (2001). Paradoxical effects of ketamine on the memory of fetuses of different ages. *Developmental Brain Research*, *127*, 71-76.

Mickley, G.A., Remmers-Roeber, D.R., Dengler, C.M., McMullen, C.A., Kenmuir, C.L., Girdler, B., et al. (2002). Simple behavioral methods to assess the effect of drugs or toxins on sensory experience. *Journal of Neuroscience Research Methods*, *115*, 85-92.

Mickley, G.A., Kenmuir, C.L., & Remmers-Roeber, D. (2003) Mentoring undergraduate students in neuroscience research outside of the classroom setting: A model system at Baldwin-Wallace College. *Journal of Undergraduate Neuroscience Education*, *1 (2)*, A28-A35.

Mickley, G.A., Kenmuir, C.L., Dengler-Crish, C.M., McMullen, C., McConnell, A.M., & Valentine, E. (2004). Repeated exposures to gustatory stimuli produce habituation or positive contrast effects in perinatal rats. *Developmental Psychobiology*, *44*, 176-188.

Mickley, G.A., Kenmuir, C.L., McMullen, C. A., Yocom, A. M., Valentine, E. L., Dengler-Crish, C.M., et al. (2004). Dynamic processing of taste aversion extinction in the brain. *Brain Research*, *1061 (1)*, 79-89.

Mickley, G.A., Kenmuir, C.L., McMullen, C., Snyder, A., McConnell, A.M., Likins-Fowler, D. et al. (2004). Long-term age-dependent behavioral changes following a single episode of fetal N-methyl-D-Aspartate (NMDA) receptor blockade. *BMC Pharmacology*, *4*, 28.

Mickley, G.A., Kenmuir, C.L., Yocom, A.M., Wellman, J.A., & Biada, J.M. (2005). A role for prefrontal cortex in extinction of a conditioned taste aversion. *Brain Research*, *1051*, 176-182.

Mickley, G.A., Biada, J.M., Kenmuir, C.L., Yocom, A.M., Wellman, J.A., Hoxha, N., & Hoxha, Z. (2005). Ketamine enhances positive contrast effects (PCEs) and up-regulates N-Methyl-D-Aspartate (NMDA) NR2B receptors in fetal rats. (Abstract Viewer, Society for Neuroscience Program No. 882.10). Abstract retrieved January 9, 2008, from http://sfn.scholarone.com/itin2005/main.html?new_page_id=126&abstract_id=5875&p_num=882.10&is_tech=0

Mickley, G.A., Hoxha, Z., Bacik, S., Kenmuir, C.L., Wellman, J.A., Biada, J.M., et al. (2007). Spontaneous recovery of a conditioned taste aversion differentially alters extinction-induced changes in c-Fos protein expression in rat amygdala and neocortex. *Brain Research*, *1152*, 139-157.

Nolan, L.J., McCaughey, S.A., Giza, B. K., Rhinehart-Doty, J.A., Smith, J.C., & Scott, T.R. (1997). Extinction of a conditioned taste aversion in rats: I. Behavioral effects. *Physiology and Behavior*, *61*, 319-323.

Pavlov, I.P. (1927). *Conditioned reflexes.* (G.V. Anrep, Trans.). London, England: Oxford

　　　University Press.

Rachman, S., & Lopatke, C. (1988). Return of fear: underlearning and overlearning. *Behaviour*

　　　Research and Therapy, *26*, 99-104.

Rauhut, A.S., Thomas, B.L., & Ayres, J.J.B. (2001). Treatments that weaken Pavlovian
　　　conditioned fear and thwart its renewal in rats: Implications for treating human
　　　phobias. *Journal of Experimental Psychology: Animal Behavior Processes, 27,* 99-
　　　114.

Smotherman, W.P., & Robinson, S.R. (1985). The rat fetus in its environment: Behavioral

　　　adjustments to novel, familiar, aversive, and conditioned stimuli presented *in utero*.

　　　Behavioral Neuroscience, *99*, 521-530.

Smotherman, W.P., & Robinson, S.R. (1990). The prenatal origins of behavioral organization.

　　　Psychological Science, *1*, 97-106.

Smotherman, W.P., & Robinson, S.R. (1991). Accessibility of the rat fetus for psychobiological

　　　investigation. In H.N. Shair, G.A. Barr, & M.A. Hoffer (Eds.), *Developmental*

　　　psychobiology: New methods and changing concepts. (pp 148-163). New York: Oxford

　　　University Press.

Wagner, A.R., & Rescorla, R.A. (Eds.). (1974). *Inhibition in Pavlovian conditioning.* London:

　　　Academic Press.

Yamamoto, T., & Fujimoto, Y. (1991). Brain mechanisms of taste aversion learning in the rat.

　　　Brain Research Bulletin, *27*, 403-406.

Yamamoto, T., Shimura, T., Sako, N., Yasoshima, Y., & Sakai, N. (1994). Neural substrates for

　　　conditioned taste aversion in the rat. *Behavioural Brain Research*, *65*,123-137.