

ISSN 0889-3667
IJCP 22(3)127-187(2009)

International Journal of Comparative Psychology



Published by the

International Society for Comparative Psychology

Stan Kuczaj, *Editor*

Special Issue on Comparative Neuroscience of Emotion
Mauricio R. Papini, *Guest Editor*

2009, Volume 22, Number 3

Editor:

Stan Kuczaj
Department of Psychology
University of Southern Mississippi
118 College Drive #5025
Hattiesburg, MS 39406, U.S.A.
s.kuczaj@usm.edu

Associate Editor:

Lauren Highfill
Department of Psychology
Eckerd College
4200 54th Avenue South
St. Petersburg, FL 33711, U.S.A
highfile@eckerd.edu

Editorial Advisory Board:

Michael J. Beran, *Georgia State University, U.S.A.*
Aaron P. Blaisdell, *University of California at Los Angeles, U.S.A.*
Daniela Brunner, *PsychoGenics and Columbia University, U.S.A.*
Jonathon D. Crystal, *University of Georgia, U.S.A.*
Nancy K. Dess, *Occidental College, U.S.A.*
Fabiana Kubke, *University of Auckland, New Zealand*
Suzanne E. MacDonald, *York University, Canada*
Lori Marino, *Emory University, U.S.A.*
Helena Matute, *Deusto University, Spain*
Roger Mellgren, *University of Texas at Arlington, U.S.A.*
Alba E. Mustaca, *University of Buenos Aires, Argentina*
Sadahiko Nakajima, *Kwansei Gakuin University, Japan*
Sergio M. Pellis, *University of Lethbridge, Canada*
Irene Pepperberg, *Harvard University, U.S.A.*
Steve Reilly, *University of Illinois at Chicago, U.S.A.*
Todd R. Schachtman, *University of Missouri, U.S.A.*
Roger K. R. Thompson, *Franklin and Marshall College, U.S.A.*
Masaki Tomonaga, *Kyoto University, Japan*

Editorial Assistants:

Kelly Caffery, Kristina Horback, Lauren Miller

International Journal of Comparative Psychology

2009, Volume 22, Number 3

Copyright © 2009 by the International Society for Comparative Psychology

- 127 Chronic pain, memory, and injury: Evolutionary clues from snail and rat
nociceptors
Edgar T. Walters
- 141 Incentive relativity and the specificity of reward expectations in honey
bees
Daniel A. Wiegmann and Brian H. Smith
- 153 The roles of endogenous opioids in fear learning
Gavan P. McNally
- 170 Role of opioid receptors in incentive contrast
Mauricio R. Papini
-

The International Journal of Comparative Psychology is sponsored by the International Society for Comparative Psychology, an affiliate of the International Union of Psychological Sciences. In consonance with the goals and activities of the Society, it publishes studies on the evolution and development of behavior, broadly defined, and in all animal species; on the use of the comparative method for the understanding of behavioral processes; and the examination of the adequacy of psychological and evolutionary theories. It accepts for review articles that deal with historical and contemporary relationships between humans and other animals that elucidate problems of ecological and behavioral processes in resource management that explicate fundamental concepts about human evolution, and that present research results and develop theories about the development and evolution of behavior. The Editor is elected by the Operations Committee of the Society. The Editorial Advisory Board is appointed by the Editor and the Operations Committee.

Manuscripts can only be submitted electronically (MS Word, preferred). The *IJCP* adheres to APA format (see inside back cover for further details).

Copyright © 2009 by the International Society for Comparative Psychology.

Published quarterly.
ISSN 0889-3667

IJCPE822(3)127-187 (2009)

Chronic Pain, Memory, and Injury: Evolutionary Clues from Snail and Rat Nociceptors

Edgar T. Walters

University of Texas Medical School at Houston, U. S. A.

The sensory component of chronic pain is amenable to comparative study and evolutionary interpretations. Pain is usually initiated by activation of nociceptors, which detect damaging stimuli. A comparison of rats and a marine snail, *Aplysia*, shows that nociceptors in each group satisfy the same functional definition and exhibit similar functional alterations, including persistent hyperexcitability and synaptic potentiation following noxious stimulation. These alterations are also associated with conventional learning and memory. Because of the ancient divergence of these lineages, some similarities probably reflect independent evolution. However, the molecular signals linked thus far to known forms of long-term neuronal plasticity represent homologous processes that are found in all metazoan cells. Persistent plasticity mechanisms now used for chronic pain and memory may have evolved originally in the earliest neurons by selective recruitment of core cell signaling and effector systems for neuronal repair, sensory compensation, and protective functions related to peripheral injury.

Few investigators of chronic pain mechanisms have paid explicit attention to evolutionary considerations. Nevertheless, interesting clues about the evolution of pain mechanisms, like the evolution of other biological phenomena, can come from comparative studies at the behavioral, cellular, and molecular levels. Pain has been defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Merskey & Bogduk, 1994). This widely accepted definition of pain leads to two distinct sets of cross-species comparisons, which differ markedly in the range of species to be considered. Sensory responses to actual or potential tissue damage (noxious stimulation) could occur, in principle, in any animal possessing a sensory system, which means virtually all living species and extinct species possessing nervous systems. Thus, some of these mechanisms may be quite primitive. On the other hand, unpleasant emotional experiences associated with actual or potential tissue damage can only be addressed effectively in species in which such emotions are likely to occur (most plausibly in animals with complex brains and extensive behavioral repertoires), which may represent a small fraction of the animal kingdom (Walters, 2008). Indeed, because emotion is defined as a subjective experience, the emotional content of pain in other species is extremely difficult (some would say impossible) to identify (Allen, 2004). Consequently, much more comparative information is available about responses to actual or potential tissue damage (nociceptive responses) than about emotional aspects of pain. Let me stress that the human pain experience, and presumably pain in some animals, normally depends upon both the nociceptive component and the emotional component. Pain that is chronic (outlasting the healing of damaged

I thank Dr. Robyn Crook for expert, critical comments on the manuscript, and the many colleagues whose research I have cited. Supported by grants from the US National Institutes of Health (NS35979) and the Christopher and Dana Reeve Foundation. Correspondence concerning this article should be addressed to Edgar T. Walters, Department of Integrative Biology and Pharmacology, University of Texas Medical School at Houston, Houston, TX 77030, U. S. A. (edgar.t.walters@uth.tmc.edu).

tissue) is surprisingly common, occurring in about 20% of the world's population (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006), and often is quite resistant to treatment. Comparative studies at the behavioral, neural, and molecular levels should lead to a better understanding of the biology of chronic pain, which might eventually help in efforts to improve therapy. In particular, comparisons of long-term alterations in nociceptive neurons and other types of neurons may shed light on mechanisms contributing to the persistence of chronic pain. Here I discuss functional and mechanistic similarities between long-term sensitization that has been described in nociceptive pathways in both molluscan and mammalian species. I then consider possible evolutionary implications of the observation that these mechanisms for persistent alterations are shared with many that are considered fundamental to conventional learning and memory.

Functional Properties of Nociceptors Are Similar in *Aplysia* and Rats

Nearly all animals exhibit defensive behavioral responses to noxious stimuli, most commonly local or generalized withdrawal, escape locomotion, and sometimes aggressive retaliation, usually followed by prolonged immobility, enhanced vigilance, and recuperative behaviors (Walters, 1994). Nociceptors are sensory neurons specialized for detecting damaging and potentially damaging stimuli, and probably are strong activators of defensive responses in most animals (although defensive responses can also be activated by threatening stimuli that do not cause tissue damage, such as olfactory, auditory, or visual stimuli). Nociceptors have been examined in only a few species, most extensively in the laboratory rat (*Rattus norvegicus*) and, among invertebrates, in a mollusc, *Aplysia californica*. This large, soft-bodied marine snail (it lacks a shell) is found commonly along the coast of southern California. *Aplysia*'s large, individually identifiable neurons have greatly facilitated the discovery of various cellular mechanisms of neuronal function and plasticity. No neurons have been investigated as intensively in *Aplysia* as the mechanosensory neurons comprising the left E (LE) cluster in the abdominal ganglion and the ventrocaudal (VC) clusters in the two pleural ganglia. These highly plastic cells have been neurons of choice to investigate basic mechanisms of learning and memory (see Kandel, 2001). Both the LE neurons, which innervate the animal's siphon (Byrne, Castellucci, & Kandel, 1974) and the VC neurons, which innervate most of the ipsilateral surface of the body (Walters et al., 2004; Walters, Byrne, Carew, & Kandel, 1983a), were found initially to have low mechanosensory thresholds and are often regarded as receptors for light touch by investigators of learning and memory (e.g., Antonov, Antonova, Kandel, & Hawkins, 2001; Barco, Bailey, & Kandel, 2006). However, the LE and VC mechanosensory neurons are properly considered nociceptors rather than low-threshold touch receptors for the following reasons.

First, under natural, unrestrained conditions light touch rarely activates LE or VC sensory neurons; the low thresholds encountered in early studies were an artifact of applying test stimuli to pieces of the body wall that were tightly pinned

to firm substrates. This effectively reduces the natural compliance of the animal's soft body (increasing the effective intensity of weak stimuli) and produces peripheral sensitization, dramatically lowering the mechanosensory threshold (Clatworthy & Walters, 1993; Ilich, Joynes, & Walters, 1994; Walters, 1987). Unless sensitized, these sensory neurons exhibit relatively high thresholds, graded responses to increasing stimulus intensities, and maximal responses to sharp, pinching stimuli that cause clear tissue damage. Second, these later studies demonstrated that the LE and VC mechanosensory neurons share a property that, among all sensory neurons, is unique to nociceptors—sensitization rather than adaptation to repeated stimulation. All other sensory neurons adapt or accommodate when repeatedly activated, whereas for at least the first several noxious stimuli, nociceptors become more sensitive and respond more vigorously to each successive stimulus. Maximal activation by noxious stimuli and sensitization by prior noxious stimulation are characteristic features of mammalian nociceptors (Ilich & Walters, 1997; Woolf & Ma, 2007). Presumably these features represent widespread adaptations to ensure that the intensity of defensive responses matches the threat posed by a noxious or quasi-noxious stimulus, as well as the increasing threat presented by repeated or prolonged noxious stimulation. Although the sharing of features, such as these, by animals as distantly related as *Aplysia* and rats suggests that they may be quite general, functional properties of nociceptive neurons need to be compared in many more animal groups to distinguish general properties of nociceptors from taxon-specific or life-style-specific properties. Various other similarities are also found between rat and *Aplysia* nociceptors, but one that has interesting evolutionary implications, and perhaps implications for chronic pain mechanisms, is the capacity of these neurons to store long-term cellular “memory” of noxious stimulation.

Nociceptors in *Aplysia* and Rats “Remember” Noxious Stimulation

Persistent alterations of mammalian nociceptors are thought to contribute to several forms of chronic pain (Cheng & Ji, 2008; Walters et al., 2008; Woolf & Ma, 2007). Long-term, memory-like changes intrinsic to mammalian nociceptors following noxious stimulation are implied by numerous observations but rarely have been tested directly. This is because events sufficiently noxious to produce long-term changes in behavior in mammals cause inflammation in the region of injury. Persistent inflammatory signals impinging on peripheral branches of nociceptors, rather than long-term alterations intrinsic to the nociceptors, are commonly assumed to drive persistent pain. However, peripheral injury and inflammation cause clear changes in gene expression within nociceptors, including an upregulation of some ion channels and growth factor receptors (Ji, Samad, Jin, Schmoll, & Woolf, 2002; Mannion et al., 1999; Waxman, Kocsis, & Black, 1994; Woolf & Costigan, 1999), which strongly indicate long-lasting alterations of nociceptor function. Furthermore, peripheral nerve injury and inflammation produce regenerative and collateral growth of nociceptor axons (Doucette & Diamond, 1987; Shea & Perl, 1985; Lu & Richardson, 1991) and a transcription-

dependent enhancement of the nociceptor's growth state that continues to be expressed *in vitro* after isolation of the neurons (Lankford, Waxman, & Kocsis, 1998; Smith & Skene, 1997). In principle, an enhanced growth state might also promote growth of new synapses within the spinal cord. Strong evidence for inflammation- or injury-induced functional (electrophysiological) changes intrinsic to nociceptors can be provided by testing neurons *in vitro*, isolated from continuing extrinsic signals. Although such tests are often made on dissociated sensory neurons, they are usually performed in acute preparations, only a few hours after dissociation. However, long-term (24 h or longer) hyperexcitability of dissociated sensory neurons following prior injury or inflammation has been documented (Ma & LaMotte, 2005; Walters et al., 2008). While such observations are among the best evidence available for intrinsic cellular "memory", they usually do not exclude the possibility that this memory only lasts long enough to amplify long-term responses initiated by the cellular trauma of dissociation (Zheng, Walters, & Song, 2007).

Aplysia sensory neurons have served as an influential model system for memory studies, so it was natural to investigate long-term as well as short-term alterations of these nociceptors produced by noxious stimulation within or close to their receptive fields. Indeed, one of the first publications about the VC tail sensory neurons described dramatic synaptic enhancement that lasted at least 75 min following noxious tail shock (Walters, Byrne, Carew, & Kandel, 1983b). It was then shown that nociceptors directly activated by tail shock display synaptic facilitation and hyperexcitability of their cell body (soma) lasting at least 24 hours (Walters, 1987). Peripheral injury, produced by either pinching and cutting the tail or by crushing the nerve that innervates the tail, produced effects on the nociceptors that lasted weeks or longer. These included peripheral axonal regeneration (Steffensen, Dulin, Walters, & Morris, 1995) and sprouting of neurites near a site of peripheral injury and within central ganglia (Steffensen et al., 1995; Billy & Walters, 1989). Functionally, peripheral injury caused a decrease in mechanosensory threshold in the damaged region (Billy & Walters, 1989; Dulin, Steffensen, Morris, & Walters, 1995), a decrease in electrical threshold of the nociceptor axon near a site of injury or intense depolarization (Weragoda, Ferrer, & Walters, 2004), and an increase in excitability (expressed as both a decrease in electrical threshold and an increase in repetitive firing) of the nociceptor soma (Gasull, Liao, Dulin, Phelps, & Walters, 2005; Ungless, Gasull, & Walters, 2002; Walters, Alizadeh, & Castro, 1991). A long-standing puzzle was why nociceptor somata demonstrate injury-induced plasticity, because in both *Aplysia* and rats the nociceptor soma is located at the end of a "blind alley", off the direct path connecting peripheral sensory receptors to the central presynaptic terminals. Recently a sensitizing function of soma hyperexcitability was revealed by showing that this hyperexcitability promotes afterdischarge in the soma when peripherally generated action potentials arrive. The afterdischarge is then relayed to other neurons in the central nervous system, amplifying the nociceptive input (Gasull et al., 2005). Finally, peripheral injury also produces synaptic facilitation (Walters et al., 1991), although it is not yet known whether the synaptic effect is intrinsic to

the nociceptor or due also or instead to other changes in the neural circuit, such as alterations in the postsynaptic neuron. As is true for the regenerative growth of mammalian nociceptors, injury-induced growth of *Aplysia* nociceptors demonstrates that at least some of the observed changes are intrinsic to the nociceptors rather than a reflection of continuing extrinsic modulation. Furthermore, an intrinsic set of mechanisms for hyperexcitability in *Aplysia* nociceptors is demonstrated by the finding that long-term hyperexcitability can be produced directly in isolated, dissociated neurons by injuring their neurites (Ambron, Zhang, Gunstream, Povelones, & Walters, 1996; Bedi, Salim, Chen, & Glanzman, 1998) or transient depolarization (Kunjilwar, Fishman, Englot, O'Neil, & Walters, 2009), and is also expressed in excised ganglia-nerve preparations in low-Ca²⁺ conditions that block ongoing release of extrinsic neuromodulators (Gasull et al., 2005; Kunjilwar et al., 2009).

Functional similarities of long-term plasticity in *Aplysia* and rat nociceptors led to a clinically relevant prediction about rat nociceptors based upon patterns of adaptive plasticity in *Aplysia* nociceptors. These patterns suggested that long-term responses of nociceptors in general to severe noxious stimulation represent a switch of the nociceptor into a persistent, intrinsically maintained, hyperfunctional state. This led us to predict that some of the most persistent and intractable forms of chronic pain in mammals depend, at least in part, upon the switch of mammalian nociceptors into a persistent hyperfunctional state after intense or prolonged exposure to signals of tissue and nerve injury. We have begun to test this idea in a model of chronic pain induced by spinal cord injury in rats. This sometimes devastating and untreatable form of pain, which occurs in a majority of human patients after spinal cord injury, was not previously thought to involve changes in nociceptors (Finnerup & Jensen, 2004). Specifically, we predicted that prolonged exposure of the central axons and terminals of nociceptors to signs of tissue injury (especially inflammatory signals) within the spinal cord would lead to hyperexcitability of nociceptors that could result in persistent spontaneous activity being generated in the somata of these neurons, as well as enhanced growth of nociceptor axons, with both effects causing central sensitization of pain pathways and spontaneous pain. These predictions have received support in preliminary studies, and suggest a new target – nociceptors – for treating this particularly resistant form of chronic pain (Walters et al., 2008).

Cellular Memory of Injury in Nociceptors Shares Mechanisms With Conventional Learning and Memory

Striking similarities exist in the behavioral responses of *Aplysia* and rats (as well as many other species) to noxious stimulation, including withdrawal reflexes, escape, guarding responses, and recuperative behaviors (Walters, 1994), and even the conditioning of fear-like responses to a context associated with noxious stimulation (Walters et al., 1981). As just described, these similarities are paralleled by functional similarities in their nociceptors, even though the neural circuits of molluscs and mammals differ as much as their gross anatomy does. It

turns out that the cellular and molecular mechanisms involved in persistent changes in behavioral responses and nociceptor excitability also are similar in *Aplysia* and rats. Furthermore, these mechanisms display substantial overlap with the mechanisms thought to underlie traditional forms of learning and memory, which are under intense investigation, especially in rodents, gastropod molluscs, and *Drosophila* (Alberini, 2009; Barco et al., 2006; Margulies, Tully, & Dubnau, 2005). In nociceptors and memory circuits these mechanisms are expressed as short- and long-term neuronal alterations; specifically, enhancement of synaptic transmission (e.g., Ji, Kohno, Moore, & Woolf, 2003; Lee & Silva, 2009) and enhancement of membrane excitability (e.g., Devor, 2006; Xu & Kang, 2005). The long-term synaptic enhancement can involve growth of new synapses (Bailey & Kandel, 2008; De Roo, Klauser, Garcia, Pogliana, & Muller, 2008). In turn, these alterations are induced and sometimes maintained by the generation of numerous plasticity signals that are common to *Aplysia* sensorimotor systems, mammalian spinal sensory systems, and mammalian circuits in the hippocampus and other parts of the brain important for learning and memory. Shared plasticity signals include Ca^{2+} influx through NMDA receptor-gated channels opened during intense electrical activity (Glanzman, 2008; Ji et al., 2003; Rao & Finkbeiner, 2007), activation of cell signaling pathways by entry of Ca^{2+} or its release from intracellular stores, and by the binding of neuromodulators and growth factors to G-protein-coupled receptors and receptor tyrosine kinases (Barco et al., 2006; Ji et al., 2003; Lu, Christian, & Lu, 2008; Pezet & McMahon, 2006; Purcell & Carew, 2003). The resulting intracellular signals are highly conserved, including the second messenger, cAMP, and activated protein kinase or lipid kinase enzymes, notably PKA, PKC, ERK, and PI3K (Barco et al., 2006; Cheng & Ji, 2008; Lee & Silva, 2009; Obata & Noguchi, 2004; Sossin, 2008). Less extensive evidence suggests that cGMP and PKG (Aley, McCarter, & Levine, 1998; Lewin & Walters, 1999; Ota, Pierre, Ploski, Queen, & Schafe, 2008; Sung & Ambron, 2004; Sung, Walters, & Ambron, 2004; Zheng et al., 2007) and a protein kinase, TOR (mTOR in mammals), which promotes local protein synthesis in axons and dendrites (Casadio et al., 1999; Hu, Chen, & Schacher, 2007; Jimenez-Diaz et al., 2008; Price et al., 2007; Sossin, 2008; Weragoda et al., 2004), also contribute to both nociceptor sensitization and conventional memory. Long-term effects triggered by some of these signals require changes in gene transcription, with the transcription factor CREB playing an important role in prominent forms of long-term plasticity in the mammalian brain (Alberini, 2009; Lee & Silva, 2009) and in *Aplysia* nociceptors (Barco et al., 2006; Casadio et al., 1999; Lewin & Walters, 1999), and perhaps in mammalian nociceptors as well (Molliver, Cook, Carlsten, Wright, & McCleskey, 2002; Simonetti, Giniatullin, & Fabbretti, 2008; Tamura, Morikawa, & Senba, 2005; Teng & Tang, 2006). Thus, at the subcellular and molecular levels, the mechanisms of long-term neuronal plasticity revealed thus far seem remarkably similar when comparing molluscs to mammals, and comparing nociceptors to hippocampal neurons.

What Explains the Similarities in Mechanisms Associated with Nociceptor “Memory” and Conventional Memory in Distantly Related Animals?

The similarities reviewed above add to similarities across major phyla many have noted in various learning phenomena. Like those similarities (Papini, 2008), these additional parallels may reflect one or more of the following evolutionary relationships: far-reaching homology extending from molecular to functional levels, massive convergence, or parallel evolution. As argued by Papini for associative learning, homology across all levels can immediately be rejected because invertebrates and vertebrates diverged so long ago that specific neural circuits mediating learning and memory functions in different phyla, and (I assume) circuits mediating nociceptive functions in different phyla, probably arose independently following this early separation. For example, it is extremely unlikely that the pleural ganglia housing nociceptor somata in *Aplysia* and the dorsal root ganglia housing nociceptor somata in rats are homologous structures (although their development may well involve some homologous processes). Moreover, the functions of nociceptive systems and specialized memory systems differ, so the selection pressures shaping each type of system probably differ. These considerations indicate that some of the similarities across distantly related nociceptive systems and between nociceptive systems and specialized memory systems reflect common solutions to related problems that were arrived at independently. For example, at a functional level there are only two ways that a nociceptor can become more effective at sending information to the central nervous system: it can become more sensitive to its inputs and it can amplify its output. Thus, if, as seems likely, strong selection pressures have favored enhanced signaling effectiveness of surviving nociceptors in a region of injury (e.g., to compensate for lost sensory branches and to increase defensive responsiveness around wounds that attract predatory and parasitic attention; Walters, 1994; Weragoda et al., 2004), one would predict that either hypersensitivity (membrane hyperexcitability) or enhancement of synaptic output, or both, would be adaptations likely to appear in unrelated nociceptors subject to similar, strong selection pressures for enhanced nociceptive function after peripheral injury.

On the other hand, massive convergence of independently derived processes is unlikely to account for the large overlap in sets of cell signals critical both for long-term sensitization of nociceptors in different phyla and for long-term memory in different phyla. Indeed, at the subcellular level, the discovery of identical cell signals playing the same basic plasticity roles in each lineage and in each form of long-term alteration demonstrates that homologous, quite primitive (Ghysen, 2003), molecular processes subserve important parts of these alterations across phyla and across functionally distinct neural systems. The recent sequencing of expressed mRNAs in *Aplysia* indicates that most genes expressed in this mollusc have homologs in mammals, including genes that encode components of signal transduction and cellular regulatory pathways (Moroz et al., 2006). This advance, and the imminent sequencing of the *Aplysia* genome

(<http://www.ncbi.nlm.nih.gov/nuccore/AASC00000000.2>), will greatly facilitate molecular comparisons between *Aplysia* and other organisms. The available cellular observations indicate that both nociceptor “memory” and conventional memory in different phyla and diverse neural systems have utilized homologous cell signaling modules to trigger and maintain long-lasting neuronal alterations. In unrelated or distantly related nociceptive systems there probably has been parallel evolution – incorporating these core regulatory modules – to solve problems of sensory compensation and maintained vigilance following injury. In specialized memory systems, the same signaling modules appear to have been utilized in neuronal alterations shaped by divergent evolutionary pressures to solve problems of information storage. An interesting question is whether selection pressures evident today provide any clues about early selection pressures that shaped primitive plasticity systems that may be ancestral to those used today for diverse types of persistent neural plasticity.

Injury: A Potent Selection Pressure for Primitive Plasticity Mechanisms?

From a biological point of view, the emotional intensity and urgency of severe pain reflect the importance of injury-related selection pressures during evolution; an organism that cannot compensate for loss of sensory function after injury, or use nociceptive sensitization to reduce chances of further injury, is likely to die sooner and have less reproductive success than one that does. Sensitization around a wound that persists long enough for healing to occur appears to be highly adaptive, and is certainly a robust phenomenon in *Aplysia* and rats. Injury is particularly interesting as an evolutionary selection pressure because it should have been present at least as long as metazoans (Walters, 1994). Thus, unlike pressures to store information about the environment or about consequences of behavioral actions, which would have had little impact until neural circuits complex enough to begin to store such information had evolved, injury-related selection pressures have probably operated on neurons (or their antecedents) from the earliest stages of neural evolution. In other words, plasticity mechanisms selected as adaptive responses to injury may have evolved very early, before the appearance of forms of learning and memory requiring integration of activity in different neural pathways (e.g., associative learning). It seems likely that the earliest neurons were sensory and motor neurons, or combined sensory-motor neurons (Ward, Thomson, White, & Brenner, 1975; Westfall & Kinnamon, 1978). Early animals were quite small and lacked shells or hard exoskeletons, so the branches of primitive neurons were close to the soft body surface and exposed to peripheral trauma from inanimate, and possibly animate, sources. Thus, from the earliest stages of neural evolution, injury-related selection pressures may have been exerted directly on primitive neurons.

An implication of these considerations is that persistent neuronal plasticity mechanisms may have been selected originally in soft-bodied ancestors of most contemporary animals for their ability (1) to repair and regenerate peripheral

axonal branches, (2) to compensate for loss of sensory function within a damaged region, and 3) to reduce the chances of aggravating an injury by subsequent movements. A fourth function may have undergone selection after predation arose – sensitization around a wounded region to accelerate responses to subsequent attacks by predators or parasites attracted to the wound (Walters, 1991, 1994; Weragoda et al., 2004). The first set of mechanisms would result in regrowth of destroyed axonal branches while the second, third, and fourth sets could include hyperexcitability of surviving branches of damaged sensory neurons, hyperexcitability of the branches of nearby, undamaged sensory neurons, hyperexcitability of the soma or central branches of sensory neurons (which could amplify trains of sensory action potentials arriving from the periphery), enhanced release of neurotransmitter from central synapses of sensory neurons, and growth of new synapses from surviving sensory neurons. Again, these or similar functional changes have been observed in *Aplysia* and rat nociceptors, and also in neurons in structures, like the hippocampus, that appear specialized for learning and memory functions.

None of the molecular signals and cellular effectors associated thus far with nociceptor plasticity and with learning and memory (see above) is unique to these forms of plasticity; each has many other roles and is found in most metazoan cells. The molecular signals (e.g., second messengers, protein kinases, transcription factors) identified with neuronal plasticity to date represent parts of highly conserved, core regulatory systems (e.g., Gerhart & Kirschner, 1997), which are also involved in other processes, including development, differentiation, adaptation to different physiological conditions, and cellular responses to stress. Such signals may have become linked in primitive neurons to the stress of peripheral injury. Once linked to injury-induced plasticity in nociceptive sensory neurons, these regulatory modules could then be “co-opted” for use in other forms of neural plasticity as nervous systems evolved. Thus, while the phenomena of nociceptive behavioral sensitization in molluscs, chronic pain in mammals, and long-term memory in mammals are homoplastic at the psychological level, they may be products of parallel evolution, utilizing homologous molecular building blocks (see Papini, 2008). The linking of these building blocks to persistent changes in neuronal function might have occurred originally in response to ubiquitous injury-related selection pressures. If this linkage occurred in a common ancestor of contemporary animals, the *persistence* of these different psychological phenomena could be a homologous property. However, if such linkages occurred independently in different lineages or different types of neurons, the persistence of each form of behavioral modification would represent a homoplastic property.

In either case, contemporary nociceptors--both in vertebrates and in invertebrates--offer a special opportunity to discover fundamental mechanisms of neuronal plasticity that may prove important for understanding the persistence of long-term memory as well as chronic pain. Conversely, the hypothesis that peripheral injury was a preeminent selection pressure driving the evolution of mechanisms of neural plasticity underscores the value of using known learning and memory mechanisms to guide the search for mechanisms, in nociceptive sensory

neurons and their targets, that contribute to the persistence of some forms of chronic pain (e.g., Ji et al., 2003). More generally, an evolutionary perspective combined with an explicit comparative approach can yield novel predictions about cellular mechanisms that may contribute to clinically important problems, such as chronic pain following spinal cord injury. Such efforts are encouraged by the growing realization that evolutionary considerations can be a valuable part of biomedical research and medicine (Nesse, Stearns, & Omenn, 2006; Williams & Nesse, 1991).

References

- Alberini, C. M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiological Reviews*, *89*, 121-145.
- Aley, K. O., McCarter, G., & Levine, J. D. (1998). Nitric oxide signaling in pain and nociceptor sensitization in the rat. *Journal of Neuroscience*, *18*, 7008-7014.
- Allen, C. (2004). Animal pain. *Nous*, *38*, 617-643.
- Ambron, R. T., Zhang, X. P., Gunstream, J. D., Povelones, M., & Walters, E. T. (1996). Intrinsic injury signals enhance growth, survival, and excitability of *Aplysia* neurons. *Journal of Neuroscience*, *16*, 7469-7477.
- Antonov, I., Antonova, I., Kandel, E. R., & Hawkins, R. D. (2001). The contribution of activity-dependent synaptic plasticity to classical conditioning in *Aplysia*. *Journal of Neuroscience*, *21*, 6413-6422.
- Bailey, C. H., & Kandel, E. R. (2008). Synaptic remodeling, synaptic growth and the storage of long-term memory in *Aplysia*. *Progress in Brain Research*, *169*, 179-198.
- Barco, A., Bailey, C. H., & Kandel, E. R. (2006). Common molecular mechanisms in explicit and implicit memory. *Journal of Neurochemistry*, *97*, 1520-1533.
- Bedi, S. S., Salim, A., Chen, S., & Glanzman, D. L. (1998). Long-term effects of axotomy on excitability and growth of isolated *Aplysia* sensory neurons in cell culture: Potential role of cAMP. *Journal of Neurophysiology*, *79*, 1371-1383.
- Billy, A. J., & Walters, E. T. (1989). Long-term expansion and sensitization of mechanosensory receptive fields in *Aplysia* support an activity-dependent model of whole-cell sensory plasticity. *Journal of Neuroscience*, *9*, 1254-1262.
- Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain*, *10*, 287-333.
- Byrne, J., Castellucci, V., & Kandel, E. R. (1974). Receptive fields and response properties of mechanoreceptor neurons innervating siphon skin and mantle shelf in *Aplysia*. *Journal of Neurophysiology*, *37*, 1041-1064.
- Casadio, A., Martin, K. C., Giustetto, M., Zhu, H., Chen, M., Bartsch, D., et al. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell*, *99*, 221-237.
- Cheng, J. K., & Ji, R. R. (2008). Intracellular signaling in primary sensory neurons and persistent pain. *Neurochemical Research*, *33*, 1970-1978.
- Clatworthy, A. L., & Walters, E. T. (1993). Rapid amplification and facilitation of mechanosensory discharge in *Aplysia* by noxious stimulation. *Journal of Neurophysiology*, *70*, 1181-1194.
- De Roo, M., Klauser, P., Garcia, P. M., Poglia, L., & Muller, D. (2008). Spine dynamics and synapse remodeling during LTP and memory processes. *Progress in Brain Research*, *169*, 199-207.

- Devor, M. (2006). Sodium channels and mechanisms of neuropathic pain. *Journal of Pain*, 7, S3-S12.
- Doucette, R., & Diamond, J. (1987). Normal and precocious sprouting of heat nociceptors in the skin of adult rats. *Journal of Comparative Neurology*, 261, 592-603.
- Dulin, M. F., Steffensen, I., Morris, C. E., & Walters, E. T. (1995). Recovery of function, peripheral sensitization and sensory neurone activation by novel pathways following axonal injury in *Aplysia californica*. *Journal of Experimental Biology*, 198, 2055-2066.
- Finnerup, N. B., & Jensen, T. S. (2004). Spinal cord injury pain--mechanisms and treatment. *European Journal of Neurology*, 11, 73-82.
- Gasull, X., Liao, X., Dulin, M. F., Phelps, C., & Walters, E. T. (2005). Evidence that long-term hyperexcitability of the sensory neuron soma induced by nerve injury in *Aplysia* is adaptive. *Journal of Neurophysiology*, 94, 2218-2230.
- Gerhart, J., & Kirschner, M. (1997). *Cells, embryos, and evolution: Toward a cellular and developmental understanding of phenotypic variation and evolutionary adaptability*. Malden, MA: Blackwell Publishers.
- Ghysen, A. (2003). The origin and evolution of the nervous system. *International Journal of Developmental Biology*, 47, 555-562.
- Glanzman, D. L. (2008). New tricks for an old slug: The critical role of postsynaptic mechanisms in learning and memory in *Aplysia*. *Progress in Brain Research*, 169, 277-292.
- Hu, J. Y., Chen, Y., & Schacher, S. (2007). Protein kinase C regulates local synthesis and secretion of a neuropeptide required for activity-dependent long-term synaptic plasticity. *Journal of Neuroscience*, 27, 8927-8939.
- Illich, P. A., Joynes, R. L., & Walters, E. T. (1994). Response-specific inhibition during general facilitation of defensive responses in *Aplysia*. *Behavioral Neuroscience*, 108(3), 614-623.
- Ji, R. R., Kohno, T., Moore, K. A., & Woolf, C. J. (2003). Central sensitization and LTP: Do pain and memory share similar mechanisms? *Trends in Neuroscience*, 26, 696-705.
- Ji, R. R., Samad, T. A., Jin, S. X., Schmoll, R., & Woolf, C. J. (2002). p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. *Neuron*, 36(1), 57-68.
- Jimenez-Diaz, L., Geranton, S. M., Passmore, G. M., Leith, J. L., Fisher, A. S., Berliocchi, L., Sivasubramaniam, A. K., Sheasby, A., Lumb, B. M., & Hunt, S. P. (2008). Local translation in primary afferent fibers regulates nociception. *PLoS ONE*, 3, e1961.
- Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science*, 294, 1030-1038.
- Kunjilwar, K. K., Fishman, H. M., Englot, D. J., O'Neil, R. G., & Walters, E. T. (2009). Long-lasting hyperexcitability induced by depolarization in the absence of detectable Ca²⁺ signals. *Journal of Neurophysiology*, 101, 1351-1360.
- Lankford, K. L., Waxman, S. G., & Kocsis, J. D. (1998). Mechanisms of enhancement of neurite regeneration in vitro following a conditioning sciatic nerve lesion. *Journal of Comparative Neurology*, 391, 11-29.
- Lee, Y. S., & Silva, A. J. (2009). The molecular and cellular biology of enhanced cognition. *Nature Reviews Neuroscience*, 10, 126-140.
- Lewin, M. R., & Walters, E. T. (1999). Cyclic GMP pathway is critical for inducing long-term sensitization of nociceptive sensory neurons. *Nature Neuroscience*, 2, 18-23.
- Lu, X., & Richardson, P. M. (1991). Inflammation near the nerve cell body enhances axonal regeneration. *Journal of Neuroscience*, 11, 972-978.

- Lu, Y., Christian, K., & Lu, B. (2008). BDNF: A key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiology of Learning and Memory*, *89*, 312-323.
- Ma, C., & LaMotte, R. H. (2005). Enhanced excitability of dissociated primary sensory neurons after chronic compression of the dorsal root ganglion in the rat. *Pain*, *113*, 106-112.
- Mannion, R. J., Costigan, M., Decosterd, I., Amaya, F., Ma, Q. P., Holstege, J. C., et al. Ji, R. R., Acheson, A., Lindsay, R. M., Wilkinson, G. A., & Woolf, C. J. (1999). Neurotrophins: Peripherally and centrally acting modulators of tactile stimulus-induced inflammatory pain hypersensitivity. *Proceedings of the National Academy of Science U S A*, *96*, 9385-9390.
- Margulies, C., Tully, T., & Dubnau, J. (2005). Deconstructing memory in *Drosophila*. *Current Biology*, *15*, R700-13.
- Merskey, H., & Bogduk, N. (1994). Part III: Pain Terms, A Current List with Definitions and Notes on Usage. In H. Merskey & N. Bogduk (Eds.), *Classification of chronic pain, IASP task force on taxonomy* (2nd ed., pp. 209-214). Seattle, WA: IASP Press.
- Molliver, D. C., Cook, S. P., Carlsten, J. A., Wright, D. E., & McCleskey, E. W. (2002). ATP and UTP excite sensory neurons and induce CREB phosphorylation through the metabotropic receptor, P2Y2. *European Journal of Neuroscience*, *16*, 1850-1860.
- Moroz, L. L., Edwards, J. R., Puthanveetil, S. V., Kohn, A. B., Ha, T., Heyland, A., et al. Knudsen, B., Sahni, A., Yu, F., Liu, L., Jezzini, S., Lovell, P., Iannuccilli, W., Chen, M., Nguyen, T., Sheng, H., Shaw, R., Kalachikov, S., Panchin, Y. V., Farmerie, W., Russo, J. J., Ju, J., & Kandel, E. R. (2006). Neuronal transcriptome of *Aplysia*: Neuronal compartments and circuitry. *Cell*, *127*, 1453-1467.
- Nesse, R. M., Stearns, S. C., & Omenn, G. S. (2006). Medicine needs evolution. *Science*, *311*, 1071.
- Obata, K., & Noguchi, K. (2004). MAPK activation in nociceptive neurons and pain hypersensitivity. *Life Sciences*, *74*(21), 2643-2653.
- Ota, K. T., Pierre, V. J., Ploski, J. E., Queen, K., & Schafe, G. E. (2008). The NO-cGMP-PKG signaling pathway regulates synaptic plasticity and fear memory consolidation in the lateral amygdala via activation of ERK/MAP kinase. *Learning and Memory*, *15*, 792-805.
- Papini, M. (2008). *Comparative psychology* (2nd ed.). New York: Psychology Press.
- Pezet, S., & McMahon, S. B. (2006). Neurotrophins: Mediators and modulators of pain. *Annual Review of Neuroscience*, *29*, 507-538.
- Price, T. J., Rashid, M. H., Millecamps, M., Sanoja, R., Entrena, J. M., & Cervero, F. (2007). Decreased nociceptive sensitization in mice lacking the fragile X mental retardation protein: Role of mGluR1/5 and mTOR. *Journal of Neuroscience*, *27*, 13958-13967.
- Purcell, A. L., & Carew, T. J. (2003). Tyrosine kinases, synaptic plasticity and memory: Insights from vertebrates and invertebrates. *Trends in Neuroscience*, *26*, 625-630.
- Rao, V. R., & Finkbeiner, S. (2007). NMDA and AMPA receptors: Old channels, new tricks. *Trends in Neuroscience*, *30*(6), 284-291.
- Shea, V. K., & Perl, E. R. (1985). Regeneration of cutaneous afferent unmyelinated (C) fibers after transection. *Journal of Neurophysiology*, *54*, 502-512.
- Simonetti, M., Giniatullin, R., & Fabbretti, E. (2008). Mechanisms mediating the enhanced gene transcription of P2X3 receptor by calcitonin gene-related peptide in trigeminal sensory neurons. *Journal of Biological Chemistry*, *283*, 18743-18752.

- Smith, D. S., & Skene, J. H. (1997). A transcription-dependent switch controls competence of adult neurons for distinct modes of axon growth. *Journal of Neuroscience*, *17*, 646-658.
- Sossin, W. S. (2008). Molecular memory traces. *Progress in Brain Research*, *169*, 3-25.
- Steffensen, I., Dulin, M. F., Walters, E. T., & Morris, C. E. (1995). Peripheral regeneration and central sprouting of sensory neurone axons in *Aplysia californica* following nerve injury. *Journal of Experimental Biology*, *198*, 2067-2078.
- Sung, Y. J., & Ambron, R. T. (2004). Pathways that elicit long-term changes in gene expression in nociceptive neurons following nerve injury: Contributions to neuropathic pain. *Neurological Research*, *26*, 195-203.
- Sung, Y. J., Walters, E. T., & Ambron, R. T. (2004). A neuronal isoform of protein kinase G couples mitogen-activated protein kinase nuclear import to axotomy-induced long-term hyperexcitability in *Aplysia* sensory neurons. *Journal of Neuroscience*, *24*, 7583-7595.
- Tamura, S., Morikawa, Y., & Senba, E. (2005). Up-regulated phosphorylation of signal transducer and activator of transcription 3 and cyclic AMP-responsive element binding protein by peripheral inflammation in primary afferent neurons possibly through oncostatin M receptor. *Neuroscience*, *133*, 797-806.
- Teng, F. Y., & Tang, B. L. (2006). Axonal regeneration in adult CNS neurons--signaling molecules and pathways. *Journal of Neurochemistry*, *96*, 1501-1508.
- Ungless, M. A., Gasull, X., & Walters, E. T. (2002). Long-term alteration of S-type potassium current and passive membrane properties in *Aplysia* sensory neurons following axotomy. *Journal of Neurophysiology*, *87*, 2408-2420.
- Walters, E. T. (1987). Multiple sensory neuronal correlates of site-specific sensitization in *Aplysia*. *Journal of Neuroscience*, *7*, 408-417.
- Walters, E. T. (1991). A functional, cellular, and evolutionary model of nociceptive plasticity in *Aplysia*. *Biological Bulletin*, *180*, 241-251.
- Walters, E. T. (1994). Injury-related behavior and neuronal plasticity: An evolutionary perspective on sensitization, hyperalgesia, and analgesia. *International Review of Neurobiology*, *36*, 325-427.
- Walters, E. T. (2008). Evolutionary aspects of pain. In A. Basbaum & M. C. Bushnell (Eds.), *Pain* (Vol. 5, pp. 175-184). Burlington, MA: Academic Press/Elsevier.
- Walters, E. T., Alizadeh, H., & Castro, G. A. (1991). Similar neuronal alterations induced by axonal injury and learning in *Aplysia*. *Science*, *253*, 797-799.
- Walters, E. T., Bodnarova, M., Billy, A. J., Dulin, M. F., Diaz-Rios, M., Miller, M. W., et al. . Moroz, L.L. (2004). Somatotopic organization and functional properties of mechanosensory neurons expressing sensorin-A mRNA in *Aplysia californica*. *Journal of Comparative Neurology*, *471*, 219-240.
- Walters, E. T., Byrne, J. H., Carew, T. J., & Kandel, E. R. (1983a). Mechanoafferent neurons innervating tail of *Aplysia*. I. Response properties and synaptic connections. *Journal of Neurophysiology*, *50*, 1522-1542.
- Walters, E. T., Byrne, J. H., Carew, T. J., & Kandel, E. R. (1983b). Mechanoafferent neurons innervating tail of *Aplysia*. II. Modulation by sensitizing stimulation. *Journal of Neurophysiology*, *50*, 1543-1559.
- Walters, E. T., Carew, T. J., & Kandel, E. R. (1981). Associative Learning in *Aplysia*: Evidence for conditioned fear in an invertebrate. *Science* *211*, 504-506.
- Walters, E. T., Fishman, H. M., Yang, Q., Du, J., Bedi, S. S., Carlton, S. M. & Grill, R.J. (2008). Spontaneous activity in small DRG neurons following spinal cord injury may contribute to chronic neuropathic pain. *Society for Neuroscience Abstract Viewer/Itinerary Planner, Program 368.21*.

- Ward, S., Thomson, N., White, J. G., & Brenner, S. (1975). Electron microscopical reconstruction of the anterior sensory anatomy of the nematode *Caenorhabditis elegans*. *Journal of Comparative Neurology*, *160*, 313-337.
- Waxman, S. G., Kocsis, J. D., & Black, J. A. (1994). Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. *Journal of Neurophysiology*, *72*, 466-470.
- Weragoda, R. M., Ferrer, E., & Walters, E. T. (2004). Memory-like alterations in *Aplysia* axons after nerve injury or localized depolarization. *Journal of Neuroscience*, *24*, 10393-10401.
- Westfall, J. A., & Kinnamon, J. C. (1978). A second sensory--motor--interneuron with neurosecretory granules in *Hydra*. *Journal of Neurocytology*, *7*, 365-379.
- Williams, G. C., & Nesse, R. M. (1991). The dawn of Darwinian medicine. *Quarterly Review of Biology*, *66*, 1-22.
- Woolf, C. J., & Costigan, M. (1999). Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proceedings of the National Academy of Science U S A*, *96*(14), 7723-7730.
- Woolf, C. J., & Ma, Q. (2007). Nociceptors--noxious stimulus detectors. *Neuron*, *55*, 353-364.
- Xu, J., & Kang, J. (2005). The mechanisms and functions of activity-dependent long-term potentiation of intrinsic excitability. *Review of Neuroscience*, *16*, 311-323.
- Zheng, J. H., Walters, E. T., & Song, X. J. (2007). Dissociation of dorsal root ganglion neurons induces hyperexcitability that is maintained by increased responsiveness to cAMP and cGMP. *Journal of Neurophysiology*, *97*, 15-25.

Original received March 23, 2009.

Revision received May 21, 2009.

Accepted June 29, 2009.

Incentive Relativity and the Specificity of Reward Expectations in Honey Bees

Daniel D. Wiegmann
Bowling Green State University, U. S. A.

Brian H. Smith
Arizona State University, U. S. A.

Honey bees were trained in a proboscis extension response procedure on a high quality reward to one of two odors under one of two contexts and then on a lower quality reward under the alternative context to the alternative odor. The performance decrement induced by the reduced reward, revealed by comparisons with subjects trained continually on the lower reward, was independent of odor-context combinations or the order of experience with stimuli. In a second experiment subjects were forward or backward conditioned to a high quality reward or fed unconditionally and then trained on a low reward in a novel context to a novel odor. The observed performance decrement depended only on exposure to the high quality reward. These results suggest that incentive contrast effects arise from a simple mechanism—the comparison of a current incentive with experienced incentives—that is effectively independent of cues that signal a reward.

Crespi (1942, 1944) discovered that rats anticipate the magnitude of a reward when they are trained to run down a runway to a goal box that contains a food reward (Elliott, 1928; Zeaman, 1949). In particular, he observed that rats trained on a high reward run more slowly to the goal box if the magnitude of the reward is suddenly reduced and, importantly, that these subjects temporarily run *more slowly* to the goal box than subjects in a control group that are trained continually on the lower reward. This observation was of special importance because it appeared to contradict the postulate that incentive determines the rate at which a stimulus and a response become associated (Hergenhahn & Olson, 2001; Hull, 1943, 1952). The numerous *incentive relativity* studies inspired by this discovery revealed that many animals form reward expectations (Flaherty, 1996). Indeed, an incentive contrast effect was observed in honey bees more than three decades ago and later studies implicated, as in vertebrates, a frustration-like process induced by the reduction of a reward (Bitterman, 1976; Couvillon & Bitterman, 1980, 1984; Shinoda & Bitterman, 1987).

Incentive relativity studies on vertebrates suggest that multiple mechanisms underlie responses to shifts of a reward (Flaherty, 1996; Mackintosh, 1974; Williams, 1983). The magnitude of incentive contrast effects in these studies (i.e., the responses of subjects that experience a reward shift relative to the responses of subjects that experience the secondary reward continually, but are otherwise treated identically) reveals that reward expectations are under direct

This research was conducted while D. D. Wiegmann was on faculty improvement leave and he thanks Bowling Green State University for financial support and the School of Life Sciences, Arizona State University for their hospitality. J. Latshaw provided technical support and advice on experimental procedures. The authors thank H. C. Cromwell, M. R. Papini and two anonymous reviewers for helpful comments on an earlier draft of this paper. Correspondence concerning this article should be addressed to D. D. Wiegmann, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, U. S. A. (ddwieg@bgsu.edu).

stimulus control. In simultaneous incentive contrast experiments, where two stimuli with different schedules of reinforcement are presented alternately, the behavioral contrast that results from the transition of reinforcement schedules is more pronounced, for example, when the reinforced stimuli share many common elements (Bloomfield, 1972; Blough, 1988; Bower, 1961; Chechile & Fowler, 1973). In addition, static contextual cues—the apparatus and other background cues—may contribute to contrast effects induced by a reduction of reward (Dachowski & Brazier, 1991; Daniel, Wood, Pellegrini, Norris, & Papini, 2008; Flaherty, 1982). For example, rats trained alternately in low and high reward runways located in different rooms run more slowly to the goal box in the former runway than subjects trained on a low reward in both runways (Flaherty & Avdzej, 1976; Flaherty, Blitzer, & Collier, 1978).

Incentive contrast studies with honey bees and bumble bees similarly suggest that conditioned and static contextual stimuli are involved in the formation of reward expectations. The control of reward expectations by conditioned stimuli is evident in a study in which honey bee foragers were trained alternately to a stimulus A that contained a 50% sucrose solution reward and a stimulus B that contained a 20% sucrose solution reward and then tested a few minutes after a final exposure to B to either A or B under conditions in which both stimuli contained the low reward (Couvillon & Bitterman, 1984). In particular, subjects tested to A showed a significant disruption of consummatory behavior relative to subjects tested to B. The control of reward expectations by static contextual stimuli is implicated in studies of honey bee and bumble bee choice behavior (Greggers & Mauelshagen, 1997; Greggers & Menzel, 1993; Menzel, 2001; Waldron, Wiegmann, & Wiegmann, 2005; Wiegmann, Wiegmann, & Waldron, 2003). For example, bumble bee foragers trained to a high reward stimulus are likely to sample a novel stimulus that contains a low quality reward if the reward contained in the familiar stimulus is reduced, but subjects temporarily fail to consume the identical, low quality reward contained in *either* stimulus (Waldron et al., 2005; Wiegmann et al., 2003).

In vertebrates behavioral responses induced by reward shifts also appear to be modulated by mechanisms that are effectively independent of stimuli that signal a reward. Incentive contrast effects in rats occur, for example, even when a radical contextual shift is coincident with a reward reduction (Flaherty, Hrabinski, & Grigson, 1990; Grigson, Spector, & Norgren, 1993). These results reveal behavioral responses that do not depend on associatively reactivated expectancies, or *cued-recall relativity*, and implicate *recognition relativity*, incentive contrast effects that arise from the ability of a subject to recognize a difference of the magnitude of incentives (Papini & Pellegrini, 2006; Daniel et al., 2008).

In this study we conducted two experiments in which we manipulated conditioned stimuli and the context of reinforcement to minimize the influence of cued-recall memory on the responses of restrained honey bees to a reduction of reward. The results of these experiments suggest that experience with food is sufficient to instantiate reward expectations and that incentive contrast effects in

honey bees, like vertebrates, are modulated by mechanisms that are effectively independent of cues that signal a reward.

Experiment 1

In a standard successive negative incentive contrast design subjects are trained first on a high reward and later under identical conditions on a lower reward. The behavior of these subjects is compared to the behavior of subjects trained continually on the lower reward. The design of this experiment involved the addition of a concomitant shift of conditioned and contextual stimuli with the reduction of reward.

In this experiment, subjects were trained in a proboscis extension response (PER) procedure in two sessions to an odor stimulus in an illuminated arena. In the initial session subjects were trained on a high or low quality reward to one of two odor stimuli under one of two light backgrounds. In the second session all subjects were trained on a low quality reward to the alternative odor under the alternative light background. Afterward, subjects were tested without reinforcement under conditions of the first and second sessions to ensure that any difference of performance in the second session between subjects that experienced a reward reduction and subjects trained continually on a low quality reward could not be attributed to satiety.

Method

Subjects

Honey bees (*Apis mellifera*) were collected individually into small glass containers when they exited from outdoor colonies maintained at Arizona State University. Individuals were cooled until they became motionless and they were then secured in a plastic harness in manner that allowed them to move their antennae and mouthparts. Bitterman, Menzel, Fietz, & Schäfer (1983) describe this procedure in detail. Subjects were allowed to acclimate undisturbed for 2-3 h and they were then tested for their responsiveness to sucrose by antennal stimulation with a 2- μ l droplet of 10% (weight percent) sucrose solution. Individuals were excluded from the study if this stimulation failed to elicit proboscis extension. In this responsiveness test prospective subjects were not allowed to consume the sucrose solution.

Apparatus

Individuals were PER conditioned in a 15 x 15 x 15 cm black acrylic arena lined on the top, bottom and sides with textured aluminum foil, which reflected light produced by two light-emitting diodes (Unitech Systems Inc., Part No. N500TBG4D) mounted on the rear floor of the arena. The two diodes emitted blue (464-475nm) or green (520-535nm) light with an intensity of 3200 mcd. The light conditions under which individuals were trained in this experiment are known to modulate the strength of learned olfactory associations (Gerber & Smith, 1998).

The front of the arena was open and subjects were placed in the center of the arena when they were trained. A 1 ml glass syringe—plunger removed—that contained a 35 x 2.5 mm piece of filter paper laden with 3 μ l of pure 1-hexanol ($\text{CH}_3(\text{CH}_2)_5\text{OH}$) or geraniol ($\text{C}_{10}\text{H}_{18}\text{O}$) was positioned on a stand in front of the arena to deliver odors to subjects. A programmable logic controller was activated a few seconds after a subject was placed into the arena. The controller regulated a valve that shunted air through the syringe and it triggered a tone to signal the appropriate time to deliver a

reward. An exhaust duct located in the back wall of the apparatus vented odors from the arena. The room was illuminated by a 25-W red light, not easily detected by honey bees (Winston, 1987).

Procedure

Subjects were classically conditioned to either 1-hexanol (X) or geraniol (R) under blue (B) or green (G) background illumination in two sessions, each of which consisted of five trials. In the first session of the experiment the reward was a 2- μ l droplet of either 10% (+) or 40% (++) sucrose solution. A trial was initiated with the placement of a subject into the arena. In each trial the odor stimulus was delivered to a subject for 4 s and a reward was delivered 3 s after the start of odor delivery. The trials within a session were separated by 5 min and 10 min separated the last trial of the first session and the first trial of the second session.

All four light and odor combinations were used in the first session in different treatment groups. Half of all subjects exposed to each light and odor combination were rewarded consistently with the low (XB+, XG+, RB+, RG+) or high (XB++, XG++, RB++, RG++) sucrose solution reward. In the second session of the experiment subjects were trained to the alternative odor under the alternative light condition on a 2- μ l droplet of 10% (+) sucrose solution reward. This design yields a total eight groups, four odor and light combinations, subdivided into groups trained on a low or high reward. Ten subjects were assigned randomly to each of the treatments.

Each subject was tested 5 min after the final trial of the second session, first under the light and odor conditions used in the initial session of the experiment and then, 5 min later, under the light and odor context experienced in the second session to ensure that any decrement of performance observed in the second session by subjects who experienced a reward reduction could not be attributed to a lack of motivation to feed. Subjects were not rewarded in either of the tests. The experimental design is summarized in Table 1.

Table 1
Summary of the Design of Experiment 1.

Session		Test	
1	2	1	2
XB++	RG+	XB	RG
XB+	RG+	XB	RG
XG++	RB+	XG	RB
XG+	RB+	XG	RB
RB++	XG+	RB	XG
RB+	XG+	RB	XG
RG++	XB+	RG	XB
RG+	XB+	RG	XB

Note: In Experiment 1 subjects were trained to either 1-hexanol (X) or geraniol (R) under blue (B) or green (G) background illumination. The symbols + and ++ identify reinforcement with a low and high reward, respectively. In tests subjects were not rewarded (indicated by a lack of a + or ++). Incentive contrast effects are revealed by comparisons of the behavior of subjects that experienced different reward levels in the first session and identical odor and light conditions in each session. These respective treatment and control groups are listed in pairs.

Statistical Analyses

In each trial and in the two tests the response of a subject was scored as a one or a zero if a subject did or did not extend its proboscis within 3 s of the initiation of odor delivery, respectively; that is, a positive response was scored only if proboscis extension occurred before the controller

triggered the tone that signaled reward delivery. The proportion of trials in which a subject extended its proboscis was recorded for each session and a repeated measures analysis of variance, with post-hoc *t* tests, was used to compare the performance of subjects over the two sessions, with the light and odor reinforcement history in the initial session as factors. The independence of the performance of subjects in the unrewarded tests and their reinforcement history in the first session was evaluated with a Fisher's exact test (Sokal & Rohlf, 1995).

Results

Figure 1 shows the acquisition curves for the two sessions. These curves reveal that subjects trained on a high quality reward in the first session of the experiment responded poorly in the second session relative to subjects trained in the initial session on a low quality reward. The repeated measures analysis of variance yielded a significant main effect for the experimental session ($F(1, 72)=14.24, p = 0.0003$) and a significant interaction between sessions and the level of reinforcement experienced by subjects in the first session ($F(1, 72) = 36.45, p < 0.0001$). The analysis indicates that subjects trained on a high quality reward responded significantly more often to the odor stimulus than subjects trained to the lower quality reward in the first session (Figure 1; $t(72) = 2.50, p = 0.0146$). But subjects trained on a high quality reward in the first session performed less well in the second session than did subjects rewarded with a low concentration sucrose solution in the first session ($t(72) = -6.59, p < 0.0001$). No other main effects or two-way or higher-order interactions were significant. The low level of responses by all subjects in the first trial of the second session also implies that subjects perceived the light-odor stimulus compounds experienced in the two sessions as distinct from one another.

In the initial test 34 of the 40 subjects that experienced a reward reduction and 30 of the 40 subjects that were trained continually on the low quality reward responded to the odor and light conditions under which they were initially trained. These response frequencies do not differ significantly (Figure 1; Fisher's exact test, $p = 0.4024$). But only 16 of the former subjects responded in the second test—under the odor and light conditions of the second session—in comparison to 32 of the subjects trained continually on the low quality reward (Fisher's exact test, $p = 0.0005$). These tests confirm that the decrement of performance in the second session by subjects who experienced a reduced reward was not due to satiety.

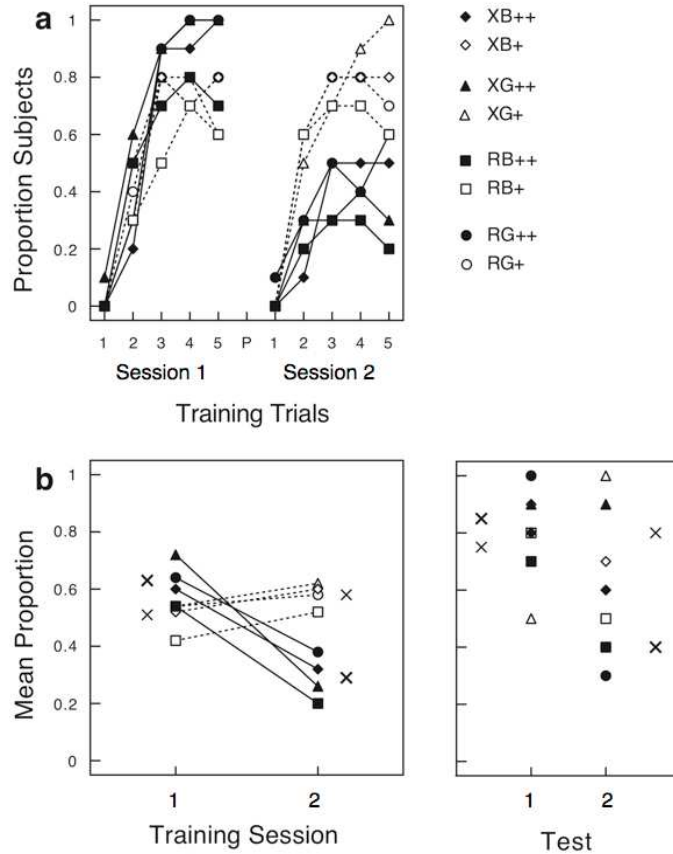


Figure 1. Acquisition curves and test responses of subjects in Experiment 1 reveal a contrast effect as a result of the reward reduction between sessions. **(a)** Proportion of subjects that responded with proboscis extension to odor delivery in each trial of the first and second sessions. The intersession interval is indicated by P. **(b)** Mean response rate for subjects in each session. Overall means for subjects trained initially on a high (++) or low (+) reward are indicated by the symbols x and x, respectively. Table 1 identifies legend symbols, which correspond to the stimuli and reward experienced by subjects in the first session.

Experiment 2

In this experiment our objective was to determine whether experience with food is sufficient to instantiate reward expectations. This experiment also involved two sessions, with subjects divided into three groups. The results of the Experiment 1 revealed that neither the combination of the olfactory stimulus and background illumination used to train subjects in the first session nor the order in which subjects were trained on particular light-odor stimulus compounds had an effect on the magnitude of the observed incentive contrast effect and for this experiment one odor-light treatment used in Experiment 1 was arbitrarily chosen to train subjects in two of the three groups. In particular, subjects in one of these

groups were forward conditioned on a high or low quality reward to geraniol under green background illumination and subjects in a second group were backward conditioned to these stimuli. Subjects in the third group were simply fed a high or low concentration of sucrose solution outside the arena. In the second session all subjects were trained to 1-hexanol under blue background illumination on a low quality reward.

An unrewarded test of the responsiveness of subjects was conducted after completion of the second session to ensure that any reduced performance in the second session by subjects that experienced a high sucrose solution concentration in the first session could not be attributed to satiety. In this test a lack of motivation to feed would be evident in a low level of responses by subjects forward conditioned on a high quality reward in the first session, relative to the performance of subjects forward conditioned initially on a low quality reward.

Method

Subjects

Individuals were collected, harnessed and tested for their responsiveness to sucrose solution as described in Experiment 1.

Apparatus

The apparatus used in this experiment was the same apparatus used in Experiment 1.

Procedure

Subjects were divided randomly into three groups. In the first session subjects in one group were PER conditioned on RG+ or RG++ as described for the first session of Experiment 1. Subjects in a second group were backward conditioned to these stimuli. These subjects were fed a 2- μ l droplet of 10% or 40% sucrose solution in the dark, outside the arena and then placed immediately into the arena, where they were treated like subjects in the former group, except that no sucrose reward was delivered (+RG, ++RG). Subjects in a third group were simply fed a 2- μ l droplet of 10% or 40% sucrose solution in the dark, outside the arena once every 5 min (+, ++). In the second session of the experiment all subjects were trained, as described in Experiment 1, in 10 trials on XB+. The final trial of the first session and the first trial of the second session were, as in Experiment 1, separated by 10 min. This design yields a total six treatments and 20 subjects were assigned to each of the treatments.

The responsiveness of each subject to geraniol under green light was tested after the second session to ensure that any decrement of performance observed in the second session by subjects fed or rewarded with a high concentration of sucrose solution in the first session could not be attributed to satiety. The interval between the final trial of the second session and the test for each subject was 5 min and in the test subjects were not rewarded. The experiment is summarized in Table 2.

Statistical Analyses

In the first session the responses of subjects that were backward or forward conditioned were scored as a one or a zero if proboscis extension did or did not occur within 3 s of the initiation of odor delivery, respectively. (In the first session subjects fed unconditionally were neither exposed to olfactory nor visual stimuli and, hence, these subjects have no scored responses). Individual responses were scored in each trial of the second session and in the test for all subjects. An analysis of variance was used to compare the performance of subjects in the first session, where the order of reward and stimuli delivery and the quality of reward served as factors. The 10 trials of the second session were divided into two equal blocks of five trials and a repeated measures analysis of variance

was used to compare the performance of subjects over the second session, with light and odor exposure and reinforcement history as factors. The independence of performance in the test and experience with stimuli and reinforcement history of subjects in the first session was evaluated with a Fisher's exact test (Sokal & Rohlf, 1995).

Table 2
Summary of the Design of Experiment 2.

Session		Test
1	2	
RG++	XB+	RG
RG+	XB+	RG
++RG	XB+	RG
+RG	XB+	RG
++	XB+	RG
+	XB+	RG

Note: Symbols are as used in Table 1. The placement of + or ++ before or after the odor and light symbols indicates whether reinforcement was delivered before or after the presentation of these paired stimuli, respectively. If incentive contrast effects occur independently of a learned association between a reward and olfactory or visual stimuli, then the responses of subjects in the second session should depend only on the concentration of sucrose solution received in the first session. Subjects were not rewarded in the test.

Results

Figure 2 shows the acquisition curves for subjects in the two sessions. The performance of subjects in the first session depended only on whether proboscis extension was forward or backward conditioned. In particular, subjects trained on RG++ or RG+ responded to the delivery of the odor stimulus with proboscis extension more often than subjects trained on ++RG or +RG ($F(1, 76) = 207.78, p < 0.0001$). The performance of subjects in this session did not depend on the level of reinforcement ($F(1, 76) = 1.351, p = 0.2487$). In addition, the influence of the manner in which subjects were trained—proboscis extension forward or backward conditioned—did not depend on the magnitude of reward ($F(1, 76) = 0.47, p = 0.4876$).

The performance of subjects in the second session reveals an incentive contrast effect in the absence of any experience with cues that signal a reward. The repeated measures analysis of variance revealed that performance of all subjects increased over the second session ($F(1, 114) = 57.83, p < 0.0001$). But the performance of subjects trained on, or fed a high concentration of sucrose solution in the first session (RG++, ++RG, ++) performed less well than subjects initially trained on, or fed a low concentration (RG+, +RG, +) of sucrose solution ($F(1, 114) = 37.58, p < 0.0001$). There was no main effect of the history of exposure to olfactory and visual stimuli on performance ($F(2, 114) = 0.33, p = 0.7042$). No two-way or higher-order interactions were significant.

The reduced performance in the second session by subjects trained on, or fed a high concentration of sucrose solution in the first session cannot be attributed to satiety. In the test all 20 subjects trained on RG++ and 18 of the 20 subjects trained on RG+ responded to the odor and light conditions under which they were initially trained. These response frequencies do not differ significantly (Figure 2; Fisher's exact test, $p = 0.4871$).

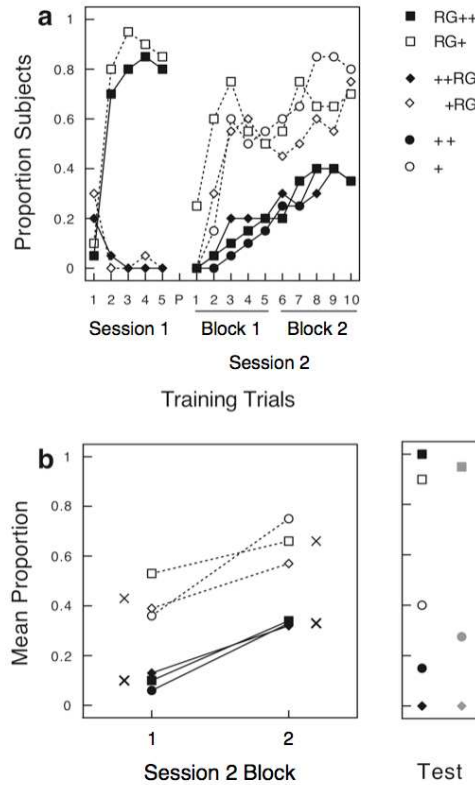


Figure 2. Acquisition curves and test responses of subjects in Experiment 2 reveal that the formation of reward expectations did not require a learned association between a reward and olfactory or contextual, visual stimuli. **(a)** Proportion of subjects that responded with proboscis extension to odor delivery in each trial of the first and second sessions. The intersession interval is indicated by P. **(b)** Mean response rate of subjects in the second session (divided into two equal blocks of five trials). Overall means for subjects that experienced a high (++) or low (+) concentration of sucrose solution in the first session are indicated by the symbols \blacksquare and \square , respectively. The gray symbols in the test results are the means of identically shaped open and solid symbols. Table 2 identifies legend symbols, which identify the treatment of subjects in the first session.

The test also reveals inhibition of responses by subjects trained in the initial session on ++RG or +RG. None of these 40 subjects responded in the test and this response frequency differs significantly from the response frequency of subjects trained in the first session on RG++ or RG+ (Fisher's exact test, $p < 0.0001$). Moreover, 11 of the 40 subjects fed + or ++ in the first session responded

in the test and this response rate is also higher than the response rate of subjects trained initially on ++RG or +RG (Fisher's exact test, $p = 0.0004$). The low rate of responses in the test by subjects trained initially on ++RG and +RG also reveals, as was observed in Experiment 1, the distinctiveness of the stimuli used in the two sessions.

Discussion

These experiments revealed that a reward reduction impedes performance, even if a substantive shift of the reinforcement context—the conditioned olfactory stimulus and static contextual cues—parallels the reduction of reward. Indeed, the results suggest that the formation of reward expectations does not require learned associations between olfactory and visual stimuli that predict a reward. Experience with a high concentration of sucrose solution, whether paired with olfactory and visual stimuli or provided unconditionally, in the absence of these stimuli, induced a similar performance decrement when subjects were later trained on a lower quality reward.

Two recent studies reveal that honey bees encode reward expectations in long term memory (Gil, De Marco, & Menzel, 2007; Gil, Menzel, & De Marco, 2008). But in incentive relativity experiments that involve a short temporal interval between the terminal experience with a high reward and the reduced reward experienced under test conditions, like those we conducted, any observed contrast effects could be ascribed, potentially, to *sensory adaptation* rather than to a process in which the secondary incentive is compared to a memory of the experienced incentive (Papini & Pellegrini, 2006). The low concentration sucrose reward used in the second session of each of our experiments may, for example, have been perceived as less sweet by subjects that experienced a reward reduction due to a sensory trace of the high concentration sucrose solution that carried over between sessions. Indeed, Bitterman (1976) attributed his original observation of contrast effects in honey bees to this form of incentive relativity, which is now referred to as *sensory relativity* (Papini & Pellegrini, 2006).

More recent incentive contrast studies with honey bees reveal, however, decided evidence of a forceful disruptive process that is distinct from sensory relativity, even when high and low quality incentives are separated by time intervals of a few minutes (Couvillon & Bitterman, 1984). Honey bees trained on a high reward for an extended number of trials also show a reduced resistance to extinction relative to subjects trained over a shorter number of trials—the *overlearning extinction effect*—even when extinction trials are, likewise, conducted a few minutes after subjects are trained (Couvillon & Bitterman, 1980, 1984; Shinoda & Bitterman, 1987). These results discredit a purely sensory explanation of incentive contrast effects observed in experiments like those we conducted.

The results of our experiments with honey bees suggest that, as in vertebrates, neither conditioned nor static contextual stimuli have exclusive control over what is learned about the quality of a reward. Indeed, the control of reward expectations by contextual stimuli implicated in earlier studies with honey bees

and bumble bees and the behavior of subjects observed in this study are consistent with a simple mechanism, namely the comparison of a current incentive with an incentive experienced previously. Elucidation of the contributions of cued-recall relativity, recognition relativity and sensory adaptation to incentive contrast effects is an important objective for future studies of honey bee responses to shifts of a reward.

References

- Bitterman, M. E. (1976). Incentive contrast in honey bees. *Science*, *192*, 380-382.
- Bitterman, M. E., Menzel, R., Fietz, A., & Schäfer, S. (1983). Classical conditioning of proboscis-extension in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, *97*, 107-119.
- Bloomfield, T. M. (1972). Contrast and inhibition in discrimination learning by the pigeon: Analysis through drug effects. *Learning & Motivation*, *3*, 162-178.
- Blough, P. M. (1988). Overall and local contrast in multiple schedules: Effects of stimulus similarity and discrimination performance. *Animal Learning & Behavior*, *16*, 395-403.
- Bower, G. H. (1961). A contrast effect in differential conditioning. *Journal of Experimental Psychology*, *62*, 196-199.
- Chechile, R., & Fowler, H. (1973). Primary and secondary negative incentive contrast in differential conditioning. *Journal of Experimental Psychology*, *97*, 189-197.
- Couvillon, P. A., & Bitterman, M. E. (1980). Some phenomena of associative learning in honeybees. *Journal of Comparative Psychology*, *94*, 878-885.
- Couvillon, P. A., & Bitterman, M. E. (1984). The overlearning extinction effect and successive negative contrast in honeybees (*Apis mellifera*). *Journal of Comparative Psychology*, *98*, 100-109.
- Crespi, L. P. (1942). Quantitative variation in incentive and performance in the white rat. *The American Journal of Psychology*, *40*, 467-517.
- Crespi, L. P. (1944). Amount of reinforcement and level of performance. *Psychological Review*, *51*, 341-357.
- Dachowski, L., & Brazier, M. M. (1991). Consummatory incentive contrast: Experimental design relationships and deprivation effects. In L. Dachowski & C. F. Flaherty (Eds.), *Current topics in animal learning* (pp. 245-270). Hillsdale, NJ: Erlbaum.
- Daniel, A. M., Wood, M., Pellegrini, S., Norris, J. N., & Papini, M. R. (2008). Can contextual cues control consummatory successive negative contrast? *Learning & Motivation*, *39*, 146-162.
- Elliott, M. H. (1928). The effect of change of reward on the maze performance of rats. *University of California Publications in Psychology*, *4*, 19-30.
- Flaherty, C. F. (1982). Incentive contrast: A review of behavioral changes following shifts in reward. *Animal Learning & Behavior*, *10*, 409-440.
- Flaherty, C. F. (1996). *Incentive Relativity*. Cambridge, U.K: Cambridge University Press.
- Flaherty, C. F., & Avdzej, A. (1976). Transsituational negative contrast. *Animal Learning & Behavior*, *4*, 49-52.
- Flaherty, C. F., Blitzer, R., & Collier, G. H. (1978). Open field behaviors elicited by reward reduction. *American Journal of Psychology*, *91*, 429-443.
- Flaherty, C. F., Hrabinski, K., & Grigson, P. S. (1990). Effect of taste context and ambient context changes on successive negative contrast. *Animal Learning & Behavior*, *18*, 271-276.

- Gerber, B., & Smith, B. H. (1998). Visual modulation of olfactory learning in honeybees. *Journal of Experimental Biology*, *201*, 2213-2217.
- Gill, M., De Marco, R. J., & Menzel, R. (2007). Learning reward expectations in honeybees. *Learning & Memory*, *14*, 491-496.
- Gill, M., Menzel, R., & De Marco, R. J. (2008). Does an insect's unconditioned response to sucrose reveal expectations of reward? *PLoS ONE*, *3*, 1-5.
- Greggers, U., & Mauerlshagen, J. (1997). Matching behavior of honeybees in a multiple-choice situation: The differential effect of environmental stimuli on the choice process. *Animal Learning & Behavior*, *25*, 458-472.
- Greggers, U., & Menzel, R. (1993). Memory dynamics and foraging strategies of honeybees. *Behavioral Ecology & Sociobiology*, *32*, 17-29.
- Grigson, P. S., Spector, A. C., & Norgren, R. (1993). Microstructural analysis of successive negative contrast in free-feeding and deprived rats. *Physiology & Behavior*, *54*, 909-916.
- Hergenhahn, B. R., & Olsen, H. M. (2001). *Theories of learning*, sixth edition. Upper Saddle River, NJ: Prentice-Hall.
- Hull, C. L. (1943). *Principles of behavior: An introduction to behavior theory*. New York: Appleton-Century-Crofts.
- Hull, C. L. (1952). *A behavior system: An introduction to behavior theory concerning the individual organism*. New Haven, CT: Yale University Press.
- Mackintosh, N. J. (1974). *The psychology of animal learning*. London, UK: Academic Press.
- Menzel, R. (2001). Behavioral and neural mechanism of learning and memory as determinants of flower constancy. In L. Chittka, & J. D Thomson (Eds.), *Cognitive ecology of pollination—Animal behavior and floral evolution* (pp. 21-40). Cambridge, U. K.: Cambridge University Press.
- Papini, M. R., & Pellegrini, S. (2006). Scaling relative incentive value in consummatory behavior. *Learning & Motivation*, *37*, 357-378.
- Shinoda, A., & Bitterman, M. E. (1987). Analysis of the overlearning-extinction effect in honeybees. *Animal Learning & Behavior*, *15*, 93-96.
- Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practice of statistics in biological research*, third edition. New York: Freeman and Company.
- Waldron, F. A., Wiegmann, D. D., & Wiegmann, D. A. (2005). Negative incentive contrast induces economic choice behavior by bumble bees. *International Journal of Comparative Psychology*, *18*, 358-371.
- Wiegmann, D. D., Wiegmann, D. A., & Waldron, F. A. (2003). Effects of a reward downshift on the consummatory behavior and flower choices of bumble bee foragers. *Physiology & Behavior*, *79*, 561-566.
- Williams, B. A. (1983). Another look at contrast in multiple schedules. *Journal of the Experimental Analysis of Behavior*, *39*, 345-384.
- Winston, M. L. (1987). *The biology of the honey bee*. Cambridge, MA: Harvard University Press.
- Zeaman, D. (1949). Response latency as a function of the amount of reinforcement. *Journal of Experimental Psychology*, *39*, 466-483.

Original received February 21, 2009.

Revision received July 24, 2009.

Accepted August 11, 2009.

The Roles of Endogenous Opioids in Fear Learning

Gavan P. McNally

The University of New South Wales, Australia

The endogenous opioid peptides and their receptors play important roles in Pavlovian fear conditioning in many species, including mice, rats, and humans. These roles are best viewed as regulating the conditions for fear learning by determining the actions of predictive error on association formation. Evidence will be reviewed showing such roles for opioid receptors in ventrolateral quadrant of the midbrain periaqueductal gray (vlPAG). These roles are shared across mammalian species because many of the effects of opioid receptor manipulations on fear learning first reported in rodents have now been documented in humans.

For the past two decades Pavlovian fear conditioning has been used extensively to study neural mechanisms of emotional learning. Exposed to pairings of a conditioned stimulus (CS) such as a tone, with an aversive unconditioned stimulus (US), such as footshock, animals learn to fear the CS as indexed by expression of co-ordinated fear responses such as species-typical defense responses, potentiated startle, and increased blood pressure upon later presentations of the CS. Fear conditioning has proven a popular model for investigations into the neural substrates of emotional learning because fear is learned rapidly, often requiring only a single trial, and because conditioned fear can persist over a long period of time. Much of this research has focussed on the role of amygdala glutamatergic neurotransmission in fear learning. This focus is unsurprising given the role of this neurotransmission in synaptic plasticity and the evidence linking amygdala synaptic plasticity to fear memory formation. However, other neurotransmitters and neuropeptides as well as other brain regions are important for fear learning. These have received significantly less empirical and theoretical attention.

This paper has two aims. The first is to review roles of the endogenous opioids in fear learning. The second is to provide a theoretical framework for understanding opioid contributions to fear learning and their potential interactions with amygdala-based glutamatergic mechanisms for fear learning. The primary focus is on rodent models of fear learning, but in recent years there has been an increase in knowledge regarding opioid contributions to fear and emotional learning in humans. These recent data strongly support the conclusions derived from non-human animal studies. This comparative research is exciting not only because it reveals common neural mechanisms for fear learning across species to underscore the important place of basic research in non-human animals, but also

The work described here has been supported by grants DP0343808 and DP0877430 to Gavan McNally and by grant DP0559967 to Gavan McNally and Rick Richardson from the Australian Research Council. I thank Sindy Cole, Laura Bradfield, and Joshua Garfield for their invaluable contributions to this research and Fred Westbrook and Rick Richardson for their helpful discussions. Correspondence concerning this article should be addressed to Gavan P. McNally, School of Psychology, The University of New South Wales, Sydney, NSW, Australia. (g.mcnally@unsw.edu.au).

because it can help to identify novel approaches to the treatment of disorders of fear and anxiety in humans.

Learning in response to positive prediction errors: Opioids and fear learning

The opioid peptides are derived from post-translational modifications of four peptide precursors: preproopiomelanocortin, preproenkephalin, prodynorphin, and proorphanin. Each precursor gives rise to multiple active opioid peptides. These peptides share the common N-terminal sequence Tyr-Gly-Gly-Phe (YGGF) followed by various C-terminus extensions producing peptides ranging from 5 to 31 residues in length. The exceptions to this rule are the peptide products of proorphanin which have a Phe-Gly-Gly-Phe (FGGF) N-terminus. The major opioid peptides encoded by the precursors include β -endorphin, Met-enkephalin, Leu-enkephalin, and dynorphin. These peptides bind to at least four receptors which have been identified via molecular cloning and pharmacological studies: μ -, δ -, κ -, and ORL receptors. The peptides derived from preproopiomelanocortin, preproenkephalin, prodynorphin bind to μ -, δ -, and κ -opioid receptors whereas the peptides derived from proorphanin bind to the ORL receptor. It is worth noting that there is a complex relationship between the opioid peptides and their receptors. The important point for present purposes is that, with the exception of the orphanin family, high affinity interactions are possible between the products of each of the peptide precursor and receptor families (for review see McNally & Akil, 2002; Williams, Christie, & Manzoni, 2001).

There is consensus that opioids are critical for regulating emotional learning and memory, with especially prominent roles in regulating fear learning. For example, in their seminal studies, Fanselow and Bolles (1979) showed that systemic administrations of the opioid receptor antagonist naloxone facilitated the acquisition of context conditioned fear learning in rodents. This finding is robust and has been replicated numerous times in many different laboratories across a variety of species. Fanselow went on to show that this facilitation of context fear conditioning was mimicked by i.c.v. infusion of a naloxone or a specific mu opioid receptor antagonist (Fanselow, Calcagnetti, & Helmstetter, 1988; Fanselow et al., 1991). The facilitation of context fear learning by opioid receptor antagonism could be subject to a number of interpretations. For example it might be suggested that opioid receptor antagonists increase fear or expression of fear conditioned responses. A closely related possibility is that the antagonists increase the aversiveness of the shock US. Alternatively, it might be suggested that the antagonists have facilitatory influences on fear memory storage and so enhance consolidation of fear memories.

Recent experiments using more complex behavioral designs have attempted to identify the specific associative process controlled by opioid receptors. This research has identified a role for opioid receptors in regulating the prediction errors which cause learning. Fear learning occurs when the actual outcome of the trial (the shock US) exceeds the expected outcome (the predicted outcome derived from the associative strengths of the CSs present). That is, when

there is a positive prediction error. When the actual outcome of the trial is not different from the expected outcome there is no prediction error and fear learning is blocked. Kamin (1968) was the first to demonstrate this effect. Kamin trained rats to fear a visual CS in Stage I. In Stage II rats received compound presentations of the visual CS + an auditory CS followed by shock. Rats in a control group received the same Stage II training but no Stage I training. Kamin showed that Stage I training blocked fear learning to the auditory CS in Stage II.

We have used the blocking design to study the effects of opioid receptor antagonism on fear learning. For example, McNally et al. (2004a) trained rats to fear a distinctive context in Stage I. Fear was measured using the species-typical defense response of freezing. In Stage II rats received auditory CS – shock pairings in that context. Prior contextual fear conditioning blocked fear conditioning to the auditory CS. Injection of naloxone prior to Stage II training prevented this blocking so that fear accrued normally to the auditory CS.

McNally and Cole (2006) used a within-subjects variant of the blocking design to study the role of opioid receptors in fear learning. This design (Figure 1) involved training rats to fear CSA in Stage I. In Stage II, for all rats, a compound of CSA+CSB was paired with shock as was a compound of CSC+CSD. Rats were tested for fear to CSB (the blocked CS) and CSD (the control CS). The logic is that Stage I training of CSA will block fear learning to CSB during Stage II. By contrast, fear learning to CSC and CSD should proceed normally because neither was paired with shock in Stage I. Blocking is shown by less fear on test to CSB as compared to CSD.

Compared with a simple experiment where an opioid receptor antagonist is administered prior to fear conditioning with a single CS, there are numerous advantages to this within-subjects design for studying fear learning. The within-subjects design isolates the contribution of predictive error to fear learning. Moreover, this design studies how the antagonist affects learning about multiple CSs in the same subjects at the same time, where those CSs differ only in their predictive error during Stage II. If opioid receptor antagonists facilitate fear learning because they increase fear, the aversiveness of the US, or facilitate consolidation of fear memories, then they will not have different effects on learning about CSB and CSD. The results of McNally and Cole (2006) showed consistently that this is not the case and instead supported a prediction error account. The results showed that: 1) blocking occurs because conditioned fear to CSB was less than conditioned fear to CSD; 2) injection of naloxone prior to Stage II prevents blocking of CSB; 3) injection of naloxone had no effect on fear learning to the control stimulus CSD.

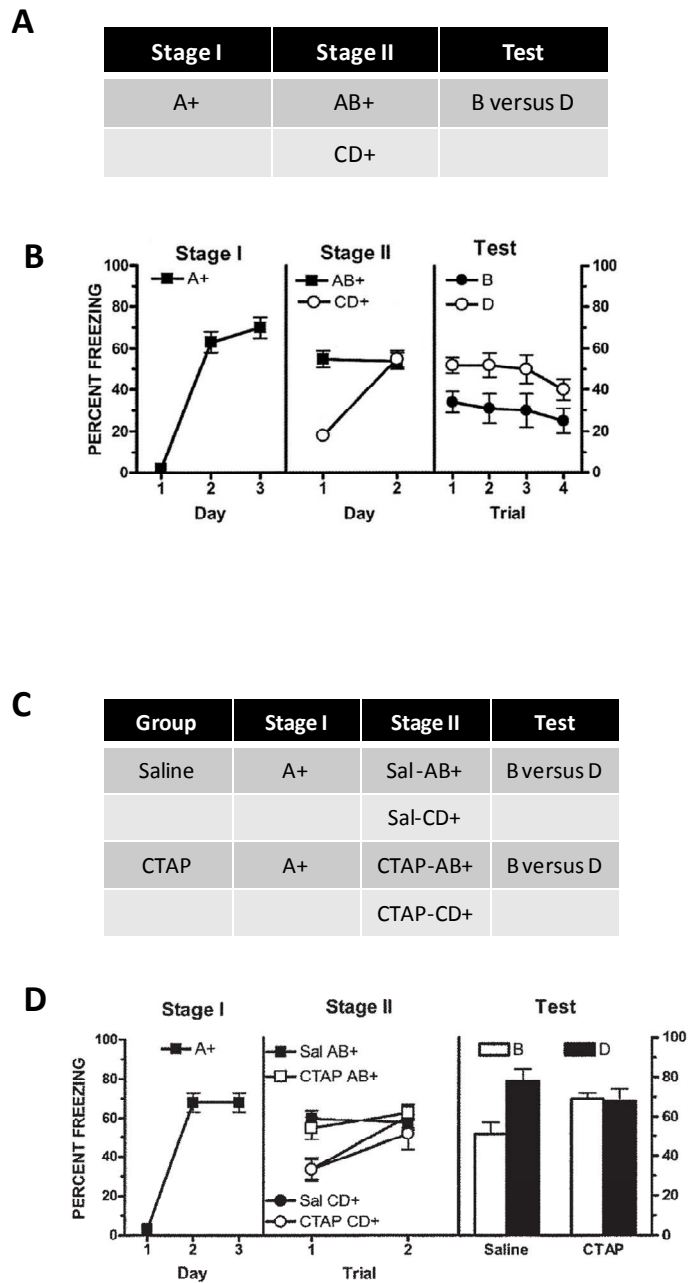


Figure 1. **A)** Behavioural design of within-subjects blocking design. **B)** Within-subjects blocking of Pavlovian fear conditioning. Stage I training of CSA blocked fear learning to CSB compared to fear learning to CSD (from McNally & Cole, 2006). **C)** Behavioural design used to investigate role of vIPAG μ opioid receptors in blocking. **D)** vIPAG infusions of a μ (CTAP) opioid receptor antagonist prevent blocking of fear conditioning (from McNally & Cole, 2006).

μ -opioid receptors in the ventrolateral quadrant of the midbrain periaqueductal gray (vlPAG) are the neuroanatomical locus for this opioid receptor regulation of fear learning. The midbrain periaqueductal gray (PAG) is an important structure for integrating defensive behavioral and autonomic responses to threats (Carrive, 1993; Fanselow, 1991; Keay & Bandler 2001, 2004). The PAG receives extensive projections from the amygdala and other forebrain structures important for learning, and it controls expression of defensive behaviors as fear CRs. The PAG is organized as a series of four longitudinal columns located dorsomedial (dm), dorsolateral (dl), lateral (l), and ventrolateral (vl) to the cerebral aqueduct that exert differential control over defensive behaviors. Both dPAG and vlPAG have been implicated in defensive responses. dPAG is important for controlling unconditioned defensive responses, whereas vlPAG is important for controlling conditioned defensive responses (Carrive, 1993). However, in addition to its well documented role in controlling fear CR expression, vlPAG plays a critical role in fear learning. We used the within-subjects blocking design to show that associative blocking of fear learning depends on endogenous opioid activation of μ -opioid receptors (Figure 1). Blocking, as measured by freezing, was prevented in a dose-dependent and neuroanatomically specific manner by vlPAG infusions of the μ -opioid receptor selective antagonist CTAP prior to Stage II training. It is worth emphasising that vlPAG infusions of CTAP did not affect the expression of fear, as measured by freezing, during Stage II. Rats infused with the μ -opioid receptor antagonist showed the same levels of fear during Stage II as control rats infused with saline. Rather, infusions of CTAP acted selectively to modulate Stage II learning by preventing the associative blocking of fear.

These results suggest that across the course of fear conditioning a fear CS acquires the ability to generate endogenous opioid signalling at μ -opioid receptors in vlPAG. This signalling acts to limit the amount of further fear learning to that CS (hence antagonists facilitate fear learning) and to block fear learning to novel stimuli conditioned in compound with that CS (hence antagonists prevent blocking). A simple, parsimonious, and theoretically coherent way to think about these results is in terms of error-correcting learning rules such as the Rescorla-Wagner model (Rescorla & Wagner, 1972). The Rescorla - Wagner model states that learning proceeds as a function of the discrepancy between the actual and expected outcomes of a conditioning trial. It provides a formal description of this discrepancy as $(\lambda - \Sigma V)$. λ is the asymptotic strength of association supported by the US, and ΣV is the summed associative strengths (V) of all conditioned stimuli present on that conditioning trial. We have suggested that opioid receptors contribute to fear learning because their activation can be specifically identified with the ΣV term in the discrepancy $(\lambda - \Sigma V)$. In other words, activation of opioid receptors contributes to encoding of the expected outcome of the conditioning trial. An opioid receptor antagonist prevents this expected outcome from regulating learning.

This approach explains why naloxone facilitates acquisition of fear learning. Across the course of conditioning the discrepancy $(\lambda - \Sigma V)$ grows smaller because the CS gains associative strength (ΣV increases). Opioid receptor

antagonists facilitate fear learning because they prevent this increase in ΣV from regulating learning on trials when the antagonist is present. In other words, opioid receptor antagonists act to maintain a large discrepancy ($\lambda - \Sigma V$) and so enhance learning. This same approach explains why opioid receptor antagonists prevent associative blocking of fear learning. Blocking occurs in the within-subjects design because the discrepancy ($\lambda - \Sigma V_A V_B$) is smaller during Stage II than the discrepancy ($\lambda - \Sigma V_C V_D$) due to the Stage I training of CSA. Opioid receptor antagonists increase the discrepancy ($\lambda - \Sigma V_A V_B$) because they prevent the V value of CSA (V_A) from regulating learning. This enables normal conditioning to CSB.

Several lines of evidence argue against the possibility that the effects of opioid receptor antagonism can be identified with an inflation of λ , or the asymptotic level of learning supported by the shock US. One such line of evidence is the fact that opioid receptor antagonists do not facilitate fear conditioning if only a single CS – US pairing (conditioning trial) is used. Rather, multiple CS – US pairings are required to detect a modulation of fear conditioning by an opioid receptor antagonist (Fanselow & Bolles, 1979). This failure of naloxone to facilitate one-trial fear conditioning is inconsistent with the possibility that opioid receptor antagonists increase λ . Instead it is consistent with the claim that these antagonist acts on a product of learning: the expected outcome, or ΣV .

Implicit in this explanation is the suggestion that fear learning which occurs following administration of an opioid receptor antagonist is different to normal fear learning. A key feature of normal fear learning is that subjects use past experience with the CS to regulate future learning about it. Opioid receptor antagonists prevent subjects from using this past experience to regulate learning. This is equivalent to suggesting that fear learning in rats treated with opioid receptor antagonists proceeds via Hebbian learning principles. For example, a key feature of Hebbian learning is that a CS will undergo increases in excitatory strength when it is paired with a US but such increases are unconstrained by predictive error, producing effectively limitless increases in fear learning. This is similar to the facilitation of fear learning by opioid receptor antagonists. Because Hebbian learning is unconstrained by predictive error, associative blocking cannot occur. Such blocking does not occur in rats treated with opioid receptor antagonists. Taken together, these data show that endogenous opioids have a critical role in regulating fear learning because they allow subjects to use past experience with the stimuli to regulate future learning about those stimuli.

It is highly unlikely that this role for opioids in predictive fear learning can be reduced simply to their role in pain modulation. Conditioned analgesia is a response to fear (Bolles & Fanselow, 1980). This analgesia can be mediated by opioid receptors (Harris, 1996). Fanselow (1998) has suggested that opioid receptor antagonists facilitate acquisition of fear because they prevent activation of descending analgesic pathways which would otherwise reduce spinal nociceptive processing of the footshock US. Several lines of evidence suggest that opioid receptors regulate predictive fear learning independently of their role in producing conditioned analgesia. For example, conditioned analgesia is frequently non-opioid (defined as insensitive to opioid receptor antagonism and lack of cross-tolerance

with morphine) (for review see Harris, 1996). Second, opioid receptor antagonists facilitate acquisition of fear to non-painful USs, such as loud noises (Cranney, 1987). Third, opioid receptor antagonists facilitate second-order fear learning which does not involve a painful US (Cicala, Azorlosa, Estall, & Grant, 1990; Cole & McNally, 2008, 2009). Fourth, opioid receptor antagonists impair fear extinction learning which involves no US (McNally & Westbrook, 2003a). Fifth, predictive fear learning is not associated with failures to detect and respond to the US, instead it is a selective alteration in learning about the affective properties of the US (Betts, Brandon, & Wagner, 1996). Thus, although opioids play an important role in conditioned analgesia, their role in regulating predictive fear learning must involve additional mechanisms to descending pain control circuits.

One such mechanism could be the large number of ascending projections from vIPAG to midline and intralaminar thalamic nuclei (Krout & Loewy, 2000). These thalamic nuclei are essential for conveying information about aversive stimuli to the amygdala, anterior cingulate cortex, prelimbic and infralimbic prefrontal cortex, as well as insula cortex. Thus, rather than viewing PAG simply as an output structure controlling fear responding via its projections to brainstem and spinal cord (e.g., LeDoux, 2000), we have suggested that PAG may be profitably viewed as part of an ascending pathway gating the transmission of aversive information to forebrain regions important for fear learning including the amygdala and prefrontal cortex (McNally, Lee, Chiem, & Choi, 2005; McNally & Cole, 2006). Recent electrophysiological data are consistent with this possibility (Johansen, Tarpley, & Blair, 2008). Lateral amygdala neurons responded strongly to a shock US but showed a diminution of this responding across the course of conditioning. This diminution was prevented by reversible inactivation of PAG, directly implicating PAG in regulating US-related activity in amygdala neurons.

The facilitation of fear learning by opioid receptor antagonism is not limited to rodents. It has also been reported in studies of fear conditioning using human subjects. Eippert, Bingel, Schoell, Yacubian, and Büchel (2008) injected human subjects with intravenous naloxone prior to fear conditioning using a discriminative (CS+/CS-) conditioning procedure with painful heat as the aversive US. Naloxone enhanced the acquisition of fear to the CS+ as measured on a reaction time task. Eippert et al. (2008) also detected diminution of US-related blood-oxygen-level-dependent (BOLD) signals in dorsal anterior cingulate across the course of conditioning which was attenuated by naloxone. Interestingly, presentations of the CS+ resulted in deactivations in rostral anterior cingulate cortex and amygdala which were prevented by naloxone. Such deactivations were observed in PAG and prevented by naloxone but these did not reach conventional levels of statistical significance. These findings are consistent not only with the behavioural data from rodents reviewed above but also with the possibility that opioid receptors in PAG exert their effects on fear learning by modulating thalamus – prefrontal cortex and thalamus – amygdala pathways.

Learning in response to negative prediction errors: Opioids and fear loss

The fear acquired through Pavlovian conditioning can be lost through learning. Fear extinction is currently the most popular animal model of this learning. In a standard extinction experiment, rats are trained to fear a CS via pairings with shock. This fear is then extinguished via repeated presentations of the CS in the absence of the US. The study of fear extinction has sparked interest among the neuroscience community due, at least in part, to its clinical importance. It is increasingly common to view anxiety-related disorders as characterised by dysregulation of fear extinction (e.g., Davis, Barad, Otto, & Southwick, 2006). Like fear acquisition, much contemporary neuroscientific research is dedicated to understanding the role of amygdala and glutamatergic neurotransmission in fear extinction learning. However, there is also evidence that the actions of opioids are central to understanding fear loss.

Systemic administrations of naloxone prior to fear extinction training prevent extinction learning. McNally and Westbrook (2003a) trained rats to fear an auditory CS via pairings with a footshock US. Fear of the CS was then extinguished via six two minute presentations of the CS. Injection of naloxone prior to extinction training impaired extinction learning as evidenced by both within-session extinction and test the following day. This impairment was dose-dependent and not due to state-dependent learning. The same injections of naloxone immediately after extinction training or prior to test for extinction had no effect on consolidation or retention of extinction. Just as the vIPAG is the key locus for opioid receptor regulation of fear acquisition, so too is it a key locus for opioid regulation of extinction. Microinjections of naloxone into vIPAG prior to extinction training produced dose-dependent and neuroanatomically specific impairments in extinction learning (McNally, Pigg, & Weidemann, 2004b). Again, μ -opioid receptors are the important subtype mediating extinction learning because infusions of μ -, but not δ -, or κ -opioid receptor antagonists into vIPAG impaired fear extinction learning (McNally et al., 2005) (Figure 2).

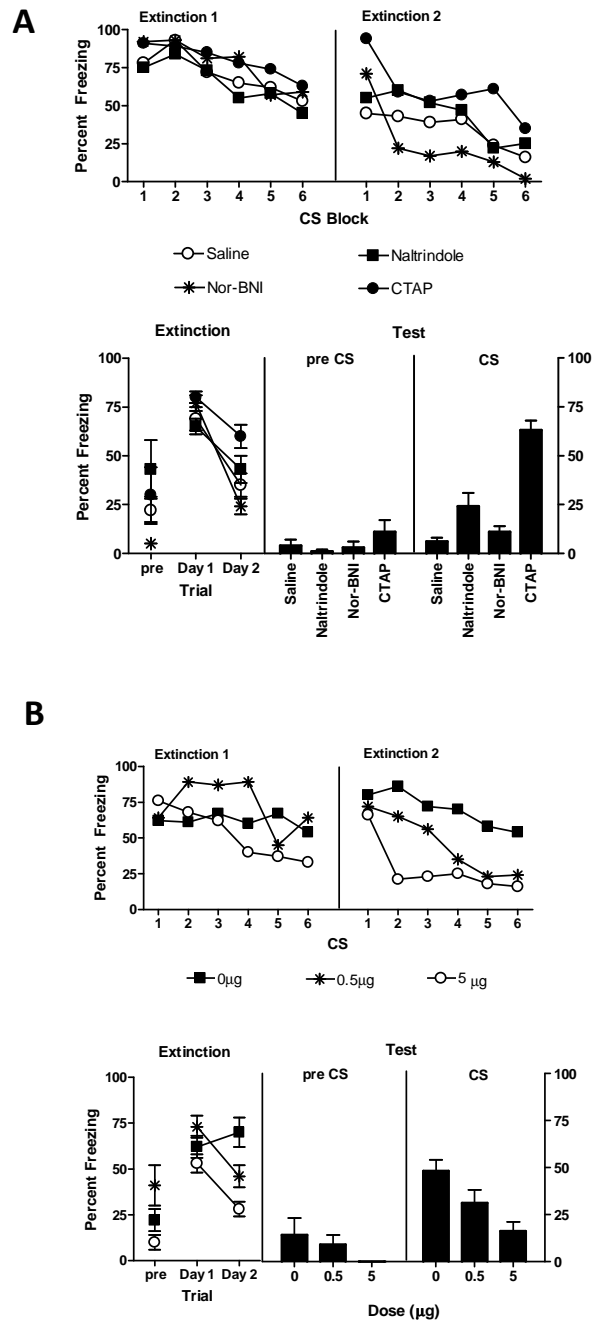


Figure 2. A) Effects of vPAG infusions of opioid receptor subtype selective antagonists on extinction of Pavlovian fear conditioning. Infusions of a μ (CTAP), but not δ (naltrindole) or κ (norBNI) opioid receptor antagonist prevents extinction (from McNally et al., 2005). B) Effects of vPAG infusions of RB101(S), an inhibitor of enkephalin catabolising enzymes, on extinction of Pavlovian fear conditioning. vPAG infusions of RB101(S) dose-dependently enhance extinction (from McNally, 2005).

Further evidence for a role for vIPAG opioids in fear extinction learning comes from experiments which have studied whether fear extinction learning can be enhanced by manipulations which enhance opioid neuromodulation. One such manipulation exploits an interesting feature of opioid biology. As mentioned previously, opioid peptides have a common YGGF sequence at their N termini which is critical for determining binding to opioid receptors (Akil et al., 1984). This YGGF sequence is also a target for proteolysis by membrane bound zinc metallopeptidases (e.g., neutral endopeptidase [NEP, neprilysin, EC 3.4.24.11] and neutral aminopeptidase [APN, EC 3.4.11.2]; Roques, 2000; Turner, 2003). These enzymes are located in the PAG (Noble et al., 2001). Peptidase inhibitors can target and inhibit the enkephalin catabolising enzymes (Roques, 2000). For example, administrations of inhibitors of enkephalin catabolism such as RB101(S) and RB3001 increase extracellular levels of enkephalin in the PAG and potentiate the behavioral effects of opioids (e.g., Roques, 2000). McNally (2005) studied whether intra-vIPAG administrations of RB101(S), an inhibitor of enkephalin catabolism, would affect extinction learning. RB101(S) permits selective augmentation of the endogenous opioid peptide signal generated during fear extinction because it reduces catabolism of opioid peptides but does not have motivational effects itself (Noble, Coric, Turcaud, Fournie-Zaluski, & Roques, 1994; Noble, Fournie-Zaluski, & Roques, 1993). Infusions of RB101(S) into vIPAG significantly augmented fear extinction learning in a dose-dependent and neuroanatomically specific manner (Figure 2).

The effects of opioid receptor antagonists on fear extinction learning cannot easily be attributed to any tendency of the antagonists to increase fear. The antagonists did not inflate the fear CR nor did they reinstate extinguished fear. Moreover, manipulations which increase fear during extinction training augment prediction error and so facilitate fear extinction learning (e.g., Leung & Westbrook, 2008) whereas opioid antagonists impair this learning. Instead, these data show that the same opioid manipulations which facilitate the acquisition of fear learning impair fear extinction learning. This may seem paradoxical from the perspective that fear extinction is a learning process. However, it is entirely consistent with the predictive learning approach to understanding fear conditioning. The conditions promoting acquisition versus the extinction of fear are different. Indeed, they are opposite. Fear acquisition depends on positive predictive error whereas fear loss depends on negative predictive error. Fear extinction is the prototypical example of negative predictive error. At the start of extinction training the discrepancy between the actual outcome of the extinction trial (no shock) and the expected outcome (shock), ($\lambda - \Sigma V$), is negative and large because $\lambda = 0$ and $\Sigma V > 0$. According to the analysis developed here, this negative prediction error should be absent in rats treated with an opioid receptor antagonist because these antagonists prevent the expected outcome (ΣV) from regulating learning on that trial. In other words, during extinction training under naloxone the discrepancy ($\lambda - \Sigma V$) is small because ΣV has been reduced by the antagonist. The behaviour of animals subjected to fear extinction under an opioid receptor

antagonist is consistent with a third principle of Hebbian learning: fear, once acquired, cannot be extinguished.

Opioid receptor activation has diverse cellular consequences, including modulation of potassium and calcium conductances, inhibition of transmitter release, and nuclear signalling (Williams et al., 2001). Of particular relevance to understanding fear extinction learning is inhibition of adenylyl cyclase and cAMP. Inhibition of adenylyl cyclase -cAMP signalling is important for extinction learning in vIPAG because extinction learning is impaired, in a dose-dependent manner, by infusions of the membrane permeable cAMP analogue 8-Br-cAMP (McNally et al., 2005). This is interesting because activation of the adenylyl cyclase-cAMP pathway is an important mechanism for synaptic plasticity and learning in hippocampus and amygdala (Kandel, 2001; Schafe, Nader, Blair, & LeDoux, 2001). The finding that reductions of cAMP signalling in vIPAG mediate extinction learning is consistent with the fact that the circumstances promoting extinction learning (negative predictive error) are the opposite to those promoting acquisition of fear (positive predictive error).

Extinction is one example of negative prediction error. Such errors are observed under other circumstances and the actions of opioids are central to the learning that occurs under these circumstances. One example is overexpectation. In a standard overexpectation design, fear of CSA and CSB is established by pairing each with shock in Stage I. In Stage II, the experimental group receives compound presentations of CSA and CSB with shock, whereas the control groups receive either additional CSA-shock pairings or no additional training. Stage II compound training of CSA and CSB reduces the amount of fear provoked by either CS (Rescorla, 1970). This occurs because during Stage II the summed predictive strengths of CSA and CSB (ΣV) exceed the amount of learning supported by the footshock US (λ). This generates negative predictive error causing loss of fear to CSA and CSB. In many ways, overexpectation is preferable to fear extinction for study of the role of predictive error in fear loss. Simple non-reinforced presentations of a fear CS during extinction training confound the contributions of a variety of associative and non-associative processes to fear loss. For example, a key procedural difference between fear acquisition and fear extinction is the presence versus absence of the US. Absence of the US during extinction has important effects on both learning and performance during extinction training independently of its role in generating the negative prediction error which contributes to extinction learning. Overexpectation designs overcome this limitation because the shock US is present during both stages of the experiment. Thus, if a neural system, neurotransmitter, or neuropeptide is important for learning not to fear in response to negative prediction error it must serve the same role in fear overexpectation as fear extinction. We have studied the role of opioids in fear overexpectation. McNally et al. (2004a) trained rats to fear CSA and CSB via separate pairings with footshock in Stage I. In Stage II, an experimental group received compound presentations of the CSA+CSB followed by footshock. A control group did not receive Stage II training. There was evidence for

overexpectation (fear loss) on test of fear to CSA. This overexpectation was prevented by injection of naloxone prior to Stage II training.

Learning versus performance: Opioids and GABA

A consistent finding from these experiments was that an opioid receptor antagonist administered prior to test did not alter expression of fear as measured by the species-typical defense response of freezing. Neither systemic nor intra-vlPAG administrations of opioid receptor antagonists, across wide dose ranges and actual levels of fear, affected expression of fear. It is widely accepted that extinction training imposes a mask on conditioned fear. This mask reduces expression of fear after extinction training. The dissociation between the effects of opioid manipulations on acquisition versus expression of fear extinction shows that opioids are important for the learning of fear extinction but not for the expression of fear after extinction training. This can be contrasted with the role for GABAergic neurotransmission in fear loss. Harris and Westbrook (1998) reported that expression of fear extinction was prevented by pre-treatment with the benzodiazepine partial inverse agonist FG7142. This identifies GABA as a possible neurochemical substrate for masking or inhibiting fear after extinction training, a possibility supported by the demonstration that fear extinction training up regulates benzodiazepine binding and gephyrin mRNA levels in the amygdala (Chhatawal, Myers, Ressler, & Davis, 2005; Heldt & Ressler, 2007).

A role for GABA in masking fear after extinction training is not specific to extinction learning. Rather, it is a product of negative predictive error. The negative prediction error during extinction training causes imposition of a GABAergic mask on fear. The evidence for this claim comes from recent studies on the mechanisms regulating expression of fear after overexpectation training. Recall that despite their procedural differences, extinction and overexpectation share a common cause (negative predictive error) and a common consequence (fear loss). Garfield and McNally (2009) trained rats to fear CSA and CSB via separate pairings with footshock in Stage I. Then, in Stage II, rats received compound pairings of CSA+CSB with footshock. This caused overexpectation of fear: there was less responding to CSA on test compared to controls which did not receive Stage II training. Garfield and McNally (2009) showed that injection of FG7142 prior to test alleviated the expression of overexpectation in a dose-dependent manner.

Taken together with the data reviewed previously there is an important difference between the effects of opioid and GABAergic manipulations on predictive fear learning. Opioids regulate *learning* in response to prediction error whereas GABA regulates *expression* of fear after negative prediction errors.

Opioids and fear loss in humans

A role for opioids in fear loss is not unique to rodents. It is also observed in humans under clinically important conditions. Exposure therapies for anxiety

disorders bear great similarity to procedures for experimental extinction of acquired fear. In both cases a feared stimulus is repeatedly presented in the absence of an aversive outcome to cause a reduction in fear. Exposure therapies have well documented efficacy for treatment of anxiety disorders (e.g., Booth & Rachman, 1992; Menzies & Clarke, 1993). Endogenous opioids mediate the therapeutic benefit of exposure therapies. Administrations of naloxone or naltrexone prior to exposure (Kozak et al., 2007; Merluzzi, Taylor, Boltwood, & Götestam, 1991) or systematic desensitization therapy (Egan, Carr, Hunt, & Adamson, 1988) for simple phobia significantly reduced treatment efficacy. Indeed, this impairment was seen regardless of whether multiple (Egan et al., 1988; Merluzzi et al., 1991) or single (Kozak et al., 2007) treatment sessions were employed indicating that it did not depend on cumulative exposure to opioid antagonists. There is important agreement between studies on fear loss via extinction training in rodents and fear loss via exposure therapy in humans: both depend critically on the actions of endogenous opioids.

The findings that opioid antagonists can impair the therapeutic efficacy of psychological treatments for anxiety disorders in humans raise the possibility that novel pharmacotherapy augmenting endogenous opioid neurotransmission may enhance the efficacy of psychological treatments for anxiety disorders. To date this possibility has not been examined. However the rodent data reviewed above using RB101(S) suggests such experiments may be worthwhile if a suitable pharmacological agent could be developed for human use. Recent data suggest that such pharmacotherapy may have additional benefit in terms of preventing the development of anxiety disorders. Bryant, Creamer, O'Donnell, Silove, and McFarlane (2008) assessed development of post-traumatic disorder (PTSD) after traumatic injury (e.g., transport accidents; assaults). Individuals had been admitted to hospital and treated with opiate analgesics. The amount of opiate exposure in the 48 hr following trauma was negatively associated with PTSD severity at 3 months. This relationship was not observed for depressive symptoms. Bryant et al. (2008) suggested that opiate exposure might have reduced later PTSD severity because it attenuated fear conditioning caused by the traumatic injury and its aftermath. This interpretation is consistent with the data reviewed here and the findings that opiates produce amnesia for fear conditioning (McNally & Westbrook, 2003b,c). This suggests that increased activity at opioid receptors in the hours following trauma may serve a protective influence against the long-term anxiogenic consequences of trauma and supports a role for opioids and their receptors in regulating human fear and anxiety.

Conclusion

The endogenous opioids acting at μ -opioid receptors in the vIPAG play important roles in regulating fear learning. μ -opioid receptor antagonists facilitate the acquisition of fear but impair the extinction, overexpectation, and blocking of fear learning. These roles are best viewed as regulating the conditions for fear learning by determining the actions of predictive error on association formation.

Manipulations which reduce μ -opioid neuromodulation in vIPAG enhance learning in response to positive predictive error and impair learning in response to negative predictive error. Conversely, manipulations which enhance μ -opioid neuromodulation in vIPAG impair learning in response to positive predictive error and facilitate learning in response to negative predictive error. Many of these effects have been observed in both non-human and human subjects. They have also been observed under clinically relevant circumstances in individuals suffering from anxiety disorders.

An important feature of the fear which is acquired in subjects treated with μ -opioid receptor antagonists is that it is divorced from the actions of predictive error. Instead this learning displays three key characteristics of Hebbian learning: apparent removal of limits on fear learning, absence of associative blocking, and absence of fear extinction. These findings show that normal opioid receptor function in vIPAG is integral to the neural mechanisms of predictive learning. During fear conditioning, vIPAG opioid receptors allow subjects to use past experience with stimuli to regulate future learning about those stimuli. We have suggested that this occurs because vIPAG opioid receptors gate transmission of information about the affective/motivational qualities of the US or its absence to amygdala, prefrontal cortex, and insular cortex via a vIPAG – midline/intralaminar thalamus pathway. In the absence of this feedback signal from vIPAG, fear learning is divorced from and proceeds independently of prediction error.

References

- Akil, H., Watson, S. J., Young, E., Lewis, M. E., Khachaturian, H., & Walker, J. M. (1984). Endogenous opioids: Biology and function. *Annual Review of Neuroscience*, 7, 223-255.
- Betts, S. L., Brandon, S. E., & Wagner, A. R. (1996). Dissociation of the blocking of conditioned eyeblink and conditioned fear following a shift in US locus. *Animal Learning & Behavior*, 24, 459-470.
- Bolles, R. C., & Fanselow, M. S. (1980). A perceptual defensive recuperative model of fear and pain. *Behavioral and Brain Sciences*, 3, 291 – 323.
- Booth, R., & Rachman, S. (1992). The reduction of claustrophobia-I. *Behavior Research & Therapy*, 30, 207-221.
- Bryant, R. A., Creamer, M., O'Donnell, M., Silove, D., & McFarlane, A. C. (2008). A study of the protective function of acute morphine administration on subsequent posttraumatic stress disorder. *Biological Psychiatry*, Dec 4. [Epub ahead of print].
- Carrive, P. (1993). The periaqueductal gray and defensive behavior: Functional representation and neuronal organization. *Behavioral Brain Research*, 58, 27-47.
- Chhatawal, J. P., Myers, K. M., Ressler, K.J., & Davis, M. (2005). Regulation of gephyrin and GABAA receptor binding within the amygdala after fear acquisition and extinction. *Journal of Neuroscience*, 25, 502-506.
- Cicala, G. A., Azorlosa, J. L., Estall, L. B., & Grant, S. J. (1990). Endogenous opioids interfere with Pavlovian second-order fear conditioning. *Psychological Science*, 1, 312-315.

- Cole, S., & McNally, G. P. (2008). Temporal-difference prediction errors and Pavlovian fear conditioning: role of NMDA and opioid receptors. *Behavioral Neuroscience*, *121*, 1043-1052.
- Cole, S., & McNally, G. P. (2009). Complementary roles for amygdala and periaqueductal gray in temporal-difference fear learning. *Learning & Memory*, *16*, 1 - 7.
- Cranney, J. (1987). Startle responding and context conditioning: Naloxone pre-treatment and stimulus intensity. *Pavlovian Journal of Biological Science*, *22*, 47-51.
- Davis, M., Barad, M., Otto, M., & Southwick, S. (2006). Combining pharmacotherapy with cognitive behavioral therapy: Traditional and new approaches. *Journal of Traumatic Stress*, *19*, 571-581.
- Egan, K. J., Carr, J. E., Hunt, D. D., & Adamson, R. (1988). Endogenous opiate system and systematic desensitization. *Journal of Consulting and Clinical Psychology*, *56*, 287-291.
- Eippert, F., Bingel, U., Schoell, E., Yacubian, J., & Büchel, C. (2008). Blockade of endogenous opioid neurotransmission enhances acquisition of conditioned fear in humans. *Journal of Neuroscience*, *28*, 5465-5472.
- Fanselow, M. S. (1998). Pavlovian conditioning, negative feedback, and blocking: mechanisms that regulate association formation. *Neuron*, *20*, 625-627.
- Fanselow, M. S. (1991). The midbrain periaqueductal gray as a coordinator of action in response to fear and anxiety. In A. Depaulis & R. Bandler (Eds.), *The midbrain periaqueductal gray matter: Functional, anatomical and neurochemical organization* (pp. 151-173). New York: Plenum.
- Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. *Journal of Comparative and Physiological Psychology*, *93*, 736-744.
- Fanselow, M. S., Calcagnetti, D. J., & Helmstetter, F. J. (1988). Peripheral versus intracerebroventricular administration of quaternary naltrexone and the enhancement of Pavlovian conditioning. *Brain Research*, *444*, 147-152.
- Fanselow, M. S., Kim, J. J., Young, S. L., Calcagnetti, D. J., DeCola, J. P., Helmstetter, F. J., & Landeira-Fernandez, J. (1991). Differential effects of selective opioid peptide antagonists on the acquisition of pavlovian fear conditioning. *Peptides*, *12*, 1033-1037.
- Garfield, J. B. B., & McNally, G. P. (2009). The effects of FG7142 on overexpectation of Pavlovian fear conditioning. *Behavioral Neuroscience*, in press.
- Harris, J. A. (1996). Descending antinociceptive mechanisms in the brainstem: Their role in the animal's defensive system. *Journal of Physiology (Paris)*, *90*, 15-25.
- Harris, J.A. & Westbrook, R.F. (1998). Evidence that GABA transmission mediates context-specific extinction of learned fear. *Psychopharmacology*, *140*, 105-115,
- Heldt, S. A., & Ressler, K. J. (2007). Training-induced changes in the expression of GABAA-associated genes in the amygdala after the acquisition and extinction of Pavlovian fear. *European Journal of Neuroscience*, *26*, 3631-3644.
- Johansen, J. P., Tarpley, J. W., & Blair, H. T. (2008). A neural pathway for instructive signalling during associative fear learning: A novel role for the midbrain periaqueductal gray. Program No. 488.1., Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, Online.
- Kamin, L. J. (1968). 'Attention-like' processes in classical conditioning. In M. R. Jones (Ed.), *Miami symposium on the prediction of behavior: Aversive stimulation* (pp. 9-33). Miami: University of Miami Press.
- Kandel, E. R. (2001). The molecular biology of memory storage: A dialogue between genes and synapses. *Science*, *294*, 1030-1038.

- Keay, K. A., & Bandler, R. (2001). Parallel circuits mediating distinct emotional coping reactions to different types of stress. *Neuroscience and Biobehavioral Reviews*, 25, 669–678.
- Keay, K. A., & Bandler, R. (2004). The periaqueductal gray. In G. Paxinos (Ed.), *The rat nervous system*, 3rd ed. (pp. 243–257). Academic Press, San Diego, CA.
- Kozak, A. T., Spates, C. R., McChargue, D. E., Bailey, K. C., Schneider, K. L., & Liepman, M. R. (2007). Naltrexone renders one-session exposure therapy less effective: a controlled pilot study. *Journal of Anxiety Disorders*, 21, 142-152.
- Krout, K. E., & Loewy, A. D. (2000). Periaqueductal gray matter projections to midline and intralaminar thalamic nuclei of the rat. *Journal of Comparative Neurology*, 424, 111-141.
- LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, 23, 155-184.
- Leung, H. T., & Westbrook, R. F. (2008). Spontaneous recovery of extinguished fear responses deepens their extinction: a role for error-correction mechanisms. *Journal of Experimental Psychology: Animal Behavior Processes*, 34, 461-474.
- McNally, G. P. (2005). Facilitation of fear extinction by midbrain periaqueductal gray infusions of RB101(S), an inhibitor of enkephalin catabolizing enzymes. *Behavioral Neuroscience*, 119, 1672-1677.
- McNally, G. P., & Akil, H. (2002). Opioid peptides and their receptors. In: K. Davis, D. Charney, J. T. Coyle, & C. Nemeroff, (Eds) *Neuropsychopharmacology: Fifth generation of progress* (pp. 35-46). New York: Lippincott, Williams, & Wilkins.
- McNally, G. P., & Cole, S. (2006). μ -opioid receptors in the midbrain periaqueductal gray regulate predictive error during Pavlovian fear conditioning. *Behavioral Neuroscience*, 120, 313-323.
- McNally, G. P., Lee, B., Chiem, J. Y., & Choi, E. A. (2005). The midbrain periaqueductal gray and fear extinction. Opioid receptor subtype and roles of cAMP, protein kinase A, and mitogen-activated protein kinase. *Behavioral Neuroscience*, 119, 1023-1033.
- McNally, G. P., Pigg, M., & Weidemann, G. (2004a). Blocking, unblocking, and overexpectation of fear: Opioid receptors regulate Pavlovian association formation. *Behavioral Neuroscience*, 118, 111-120.
- McNally, G. P., Pigg, M., & Weidemann, G. (2004b). Opioid receptors in the midbrain periaqueductal gray matter regulate the extinction of Pavlovian fear conditioning. *The Journal of Neuroscience*, 24, 6912-6919.
- McNally, G. P., & Westbrook, R. F. (2003a). Opioid receptors regulate the extinction of Pavlovian fear conditioning. *Behavioral Neuroscience*, 117, 1292-1301.
- McNally, G. P., & Westbrook, R. F. (2003b). Temporally-graded anterograde deficits in Pavlovian fear conditioning and the role of one-trial overshadowing: Effects of pre-conditioning exposures to morphine. *Journal of Experimental Psychology: Animal Behavior Processes*, 29, 222-232.
- McNally, G. P., & Westbrook, R. F. (2003c). Temporally-graded, context specific retrograde amnesia and its alleviation by context pre-exposure: Effects of post-conditioning exposures to morphine in the rat. *Journal of Experimental Psychology: Animal Behavior Processes*, 29, 30-142.
- Menzies, R. G., & Clarke, J. C. (1993). A comparison of in vivo and vicarious exposure in the treatment of childhood water phobia. *Behavior Research & Therapy*, 31, 9-15.
- Merluzzi, T. V., Taylor, C. B., Boltwood, M., & Götestam, K. G. (1991). Opioid antagonist impedes exposure. *Journal of Consulting and Clinical Psychology*, 59, 425-430.

- Noble, F., Banisadr, G., Jardinaud, F., Popovici, T., Lai-Kuen, R., Chen, H., Bischoff, L., Parsadaniantz, S. M., Fournie-Zaluski, M. C., & Roques, B. P. (2001). First discrete autoradiographic distribution of aminopeptidase N in various structures of rat brain and spinal cord using the selective iodinated inhibitor [125I]RB 129. *Neuroscience*, *105*, 479-488.
- Noble, F., Coric, P., Turcaud, S., Fournie-Zaluski, M. C., & Roques, B. P. (1994). Assessment of physical dependence after continuous perfusion into the rat jugular vein of the mixed inhibitor of enkephalin-degrading enzymes, RB 101. *European Journal of Pharmacology*, *253*, 283-287.
- Noble, F., Fournie-Zaluski, M. C., & Roques, B. P. (1993). Unlike morphine the endogenous enkephalins protected by RB101 are unable to establish a conditioned place preference in mice. *European Journal of Pharmacology*, *230*, 139-149.
- Rescorla, R. A. (1970). Reduction in the effectiveness of reinforcement after prior excitatory conditioning. *Learning & Motivation*, *1*, 372-381.
- Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black, W.F. Prokasy (Eds.), *Classical conditioning II: Current research and theory* (pp. 64-99). New York: Appleton Century Crofts.
- Roques, B. P. (2000). Novel approaches to targeting neuropeptide systems. *Trends in Pharmacological Sciences*, *21*, 475- 483.
- Schafe, G. E., Nader, K., Blair, H. T., & LeDoux, J. E. (2001). Memory consolidation of Pavlovian fear conditioning: A cellular and molecular perspective. *Trends in Neuroscience*, *24*, 540 -546.
- Turner, A. J. (2003). Exploring the structure and function of zinc metallopeptidases: Old enzymes and new discoveries. *Biochemistry Society Transactions*, *31*, 723-727.
- Williams, J. T., Christie, M. J., & Manzoni, O. (2001). Cellular and synaptic adaptations mediating opioid dependence. *Physiological Reviews*, *81*, 299-343.

Original received December 19, 2008.

Revision received February 16, 2009.

Accepted March 12, 2009.

Role of Opioid Receptors in Incentive Contrast

Mauricio R. Papini
Texas Christian University, U. S. A.

A downshift from a more preferred to a less preferred incentive leads to a transient rejection of the lower incentive. This phenomenon, known as successive negative contrast (SNC), has been reported in studies with mammals, but not with fish, amphibians, or reptiles, all showing gradual adjustments to the new incentive conditions. It is assumed that an understanding of the brain systems involved in the onset of SNC in mammals will suggest likely brain areas for a comparative analysis in nonmammalian vertebrates. Studies reviewed in this article show that opioid receptors are normally engaged during SNC, participate in the detection of the incentive downshift, play a role in SNC onset (delta receptors), and modulate recovery from SNC (kappa receptors). However, opioid receptors do not seem to be involved in the consolidation of the downshift memory. These results suggest a relationship between the evolution of the opioid system and the evolution of learning mechanisms involved in the adjustment to incentive downshifts in vertebrates.

Most animals can be viewed as open systems in behavioral interaction with the environment to obtain resources important for their survival and reproductive success (sessile animals may be cited as exceptions, e.g., sponges). Such resources are called incentives and include food, fluids, shelter, nesting locations and materials, social companions, and others. Incentives have both absolute and relative value. The absolute value of incentives is demonstrated by the basic instrumental conditioning procedure, according to which an animal modifies an existing response or acquires a new response when that response is followed by an incentive (Thorndike, 1911). The relative value of incentives is demonstrated when the behavior supported by an actual incentive depends on the value of past incentives experienced under similar conditions (Elliott, 1928).

Incentive relativity is the basis of a wide variety of phenomena grouped together under the name of incentive contrast effects (see Flaherty, 1996). This article is concerned with one such type of incentive contrast effect known as successive negative contrast (SNC). In the classic demonstration of SNC, Elliott (1928) trained two groups of rats in a complex maze to locate an incentive and measured both the time to reach the goal (latency) and the number of entries in blind alleys (errors). One group was rewarded with bran mash, a wet mixture of cereals (the large incentive, L), whereas the other was rewarded with sunflower seeds (the small incentive, S). Rats learned the correct path to the goal faster when rewarded with L than when rewarded with S, but a shift from L to S resulted in a fast-emerging behavioral disruption (Figure 1a). Notice that the incentive conditions during postshift trials were equal for both groups. A generally accepted view of SNC suggests that the behavioral disruption reflects a comparison between the current incentive and the reactivated memory of the incentive previously

The author thanks C. E. Stevens, A. M. Daniel, and one anonymous reviewer for their helpful comments on a previous version of this paper. Stan Kuczaj served as acting editor for this article. Correspondence concerning this article may be addressed to Mauricio R. Papini, Department of Psychology, Texas Christian University, Box 298920, Fort Worth, TX 76129, U. S. A. (m.papini@tcu.edu).

received in that situation (see Papini & Pellegrini, 2006). When the disparity between past and current incentive is sufficiently large, then a variety of behavioral and physiological effects are observed, including changes in aggressive behavior, glucocorticoid levels, vocalizations, and escape behavior (see Papini & Dudley, 1997). Furthermore, treatments known from other experiments to relate to emotional effects, such as administration of anxiolytics or lesions in limbic structures, also modulate SNC (see Flaherty, 1996). Thus, it is widely appreciated that, when the disparity is significant, the comparator mechanism induces a series of effects that may collectively be referred to as emotional (Flaherty, 1996). The emotion in question is frustration, here defined as an aversive internal state induced by the surprising reduction or omission of an expected incentive (see Amsel, 1992). Amsel (1992) distinguished between an unconditioned form (primary frustration, occurring second-to-minutes after a surprising downshift event) and a conditioned form (secondary frustration, occurring in anticipation of a frustrating event).

As mentioned above, comparative studies suggest that SNC may not be a general learning phenomenon, at least among vertebrates. The SNC and similar effects have been reported in honeybees (Bitterman, 1976; Couvillon & Bitterman, 1984) and bumblebees (Waldron, Wiegmann, & Wiegmann, 2005), but the analysis of these effects at a neurobiological level is almost nonexistent in invertebrates. Consequently, this review is restricted to studies involving vertebrates. Experiments with species assigned to conservative vertebrate lineages in terms of brain structure, such as bony fish (e.g., Lowes & Bitterman, 1967), amphibians (e.g., Papini, Muzio, & Segura, 1995), and reptiles (e.g., Papini & Ishida, 1994), suggest that whereas these animals discriminate different incentive magnitudes, incentive downshift leads to, at best, a gradual adjustment of behavior to the new incentive conditions (see two examples in Figure 1b,c). This is referred to as reversed SNC. Although the mechanisms underlying SNC were proposed to be unique to mammals (Papini, 2002, 2003, 2006; see Bentosela, Jakovsevic, Elgier, Mustaca, & Papini, 2009), starlings (Freidin, Cuello, & Kacelnik, 2009; although not pigeons, Papini, 1997) must be added to the list of species exhibiting this effect. Several research strategies may be implemented to determine the source of this apparent species divergence in learning mechanism. The strategy illustrated in this article is based on a levels approach to learning mechanisms designed to capture some of the most traditional approaches to the study of learning, including the behavioral tradition traced back to Thorndike (1911) and the neurobiological tradition represented by Lashley's (1929) work. Figure 2 captures this idea in terms of four levels of mechanistic analysis. At the top, the traditional behavioral analysis of learning processes, as represented by Thorndike, Tolman, Hull, and the traditions that branched from these early contributions. A psychological level of mechanistic analysis refers to such concepts as stimulus-stimulus associations, as illustrated in the figure. These ideas are "modular" in the sense that they can be applied to a variety of learning processes, including appetitive conditioning and fear conditioning. Unlike the concepts used at other levels of analysis, these terms are specific to the analysis of learning mechanisms. The neurobiological level

refers to studies involving such techniques as brain lesion (pioneered by Lashley), stimulation, and recording of neural activity in relatively large cell populations. These studies aim at identifying the circuitry involved by any given learning phenomenon. The neurochemical level refers to traditional studies involving drug manipulations, such as most of the research reviewed in this article. Pavlov (1927) pioneered these studies by assessing, for example, the effects of bromides on experimental neurosis induced by conditioning procedures and even used morphine as an unconditioned stimulus. Drugs are the main factors used to study synaptic properties related to learning mechanisms. Finally, the cell-molecular level involves factors that interfere with cellular processes involved in synaptic plasticity. Some of the earliest examples involve studies on the role of protein synthesis in long-term memory (e.g., Agranoff, Davis, & Brink, 1966; Potts & Bitterman, 1967). Notice, however, that brain areas, neurotransmitters, and cellular processes are not specific to learning mechanisms, but intervene in a wide variety of biological processes (e.g., Kandel & Abel, 1995).

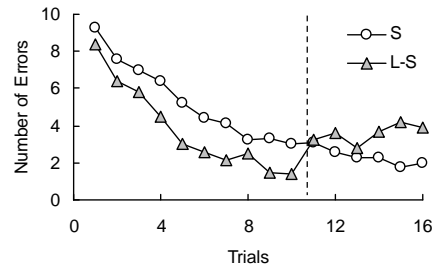
According to the approach illustrated in Figure 2, for learning phenomena in different species to be considered homologous (i.e., based on inheritance from a common ancestor), they must be based on the same mechanisms at the psychological, neurobiological, neurochemical, and cell-molecular levels. Because most progress in the understanding of mammalian SNC has been achieved in the area of neurochemical mechanisms (Flaherty, 1996), the strategy followed in the experiments described in this article aims at discovering the neurochemical systems involved in SNC onset in rats. It is hypothesized that phylogenetic changes in such systems are responsible for the evolution of mechanisms underlying SNC in vertebrates.

Role of Opioid Receptors in SNC

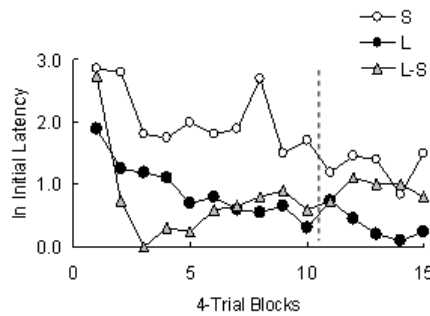
We owe most of our understanding of the neurochemical mechanisms underlying SNC to Flaherty and collaborators (see Flaherty, 1996), who used a consummatory version of this effect (cSNC). In the cSNC situation, two groups of rats receive ten 5-min-long daily trials of access to either 32% or 4% sucrose solutions (the L and S incentives, respectively), followed by five trials in which all rats are exposed to 4% sucrose. Various dependent measures have been used, including the amount of fluid intake, licking responses, and the cumulative time in contact with the sipper tube. Downshifted rats typically reject the 4% sucrose, but their behavior eventually recovers to the level of the unshifted controls only exposed to 4% sucrose. Flaherty (1996) discovered that certain drugs, such as benzodiazepine anxiolytics and ethanol, reduce cSNC on the second postshift trial (usually trial 12), but have no effect when administered before the first postshift trial (usually trial 11). This trial selectivity suggests that the mechanisms that control the triggering of cSNC and those controlling recovery from cSNC are dissociable. If the goal is to understand cSNC onset, then one would have to identify neurochemical systems that are activated selectively on trial 11. Flaherty's research failed to produce unequivocal evidence of this type of trial selectivity, but

he identified several drugs that reduced cSNC when administered before trials 11 and 12, including sodium amytal, cyproheptadine, and morphine (Flaherty, 1996). Of these three, morphine taps on a single neurochemical system, the opioid system, well known because of its role in the modulation of peripheral pain and conditioned fear, among other functions. Because of the known parallels between pain-fear and frustration (e.g., Gray, 1987; Wagner, 1969), it seemed appropriate to concentrate first on opioid receptors.

(a) SNC in Rats



(b) Reversed SNC in Pigeons



(c) Reversed SNC in Toads

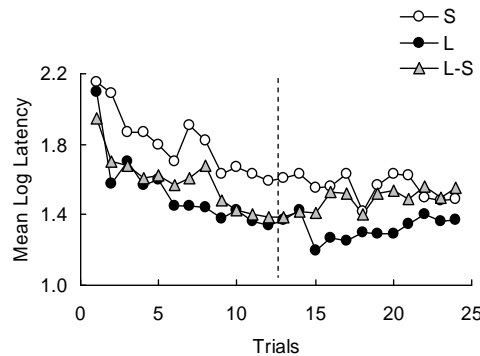


Figure 1. (a) The successive negative contrast (SNC) effect originally reported by Elliott (1928) in rats. (b) Reversed SNC effect in pigeons (Papini, 1997), and (c) in terrestrial toads (Papini, Muzio, & Segura, 1995). The dotted line marks the transition from a large (L) to a small (S) incentive. In these experiments, training involved a single trial per day. A runway was used with rats and toads, but the pigeon data were collected in a Skinner box situation. L was bran mash for rats, fifteen 45-mg food

pellets for pigeons, and 1,280 s of access to water for toads. S was sunflower seeds for rats, one 45-mg food pellet for pigeons, and 80 s of access to water for toads.

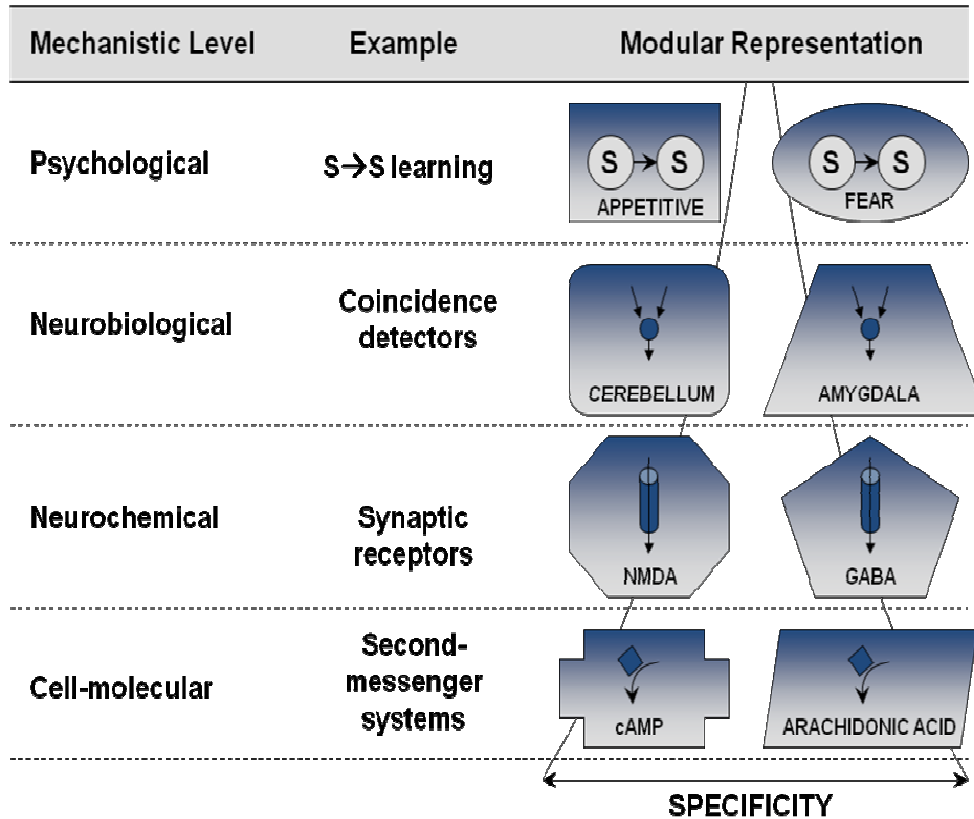


Figure 2. Four mechanistic levels of analysis of learning phenomena such as SNC (Papini, 2008). The modular representation column depicts alternative implementations of these mechanisms at each level. Modularity is implied in the possibility that any specific mechanism at one level may play a role in more than one mechanism at a higher level (e.g., NMDA receptors may be implicated in different types of learning, in different brain areas). Specificity refers to the fact that only psychological concepts are restricted in their application to explain learning phenomena. For example, cAMP is found in bacteria; being unicellular organisms, there is no synaptic plasticity in which cAMP could play a role similar to that which has been identified in animals.

The opioid system is relatively well characterized, from the genes coding for receptors and the precursors of their endogenous ligands, to the distribution and mRNA expression patterns in the rat brain (see Dreborg, Sundström, Larsson, & Larhammar, 2008; Ikeda et al., 2005; Mansour, Fox, Akil, & Watson, 1995; McNally & Akil, 2002; Sim-Selley, Vogt, Childers, & Vogt, 2003). Solutions derived from the poppy seed have been used for millennia to reduce pain induced by physical injury (Brownstein, 1993). Their active ingredient was isolated in 1806

and named morphine by Friedrich Sertürner. Of the four recognized opioid receptors, morphine has greater affinity for the mu receptor (MOR), but it also binds to the delta (DOR) and kappa (KOR) receptors, although not to the opioid receptor-like (ORL) receptor (also known as nociceptin opioid peptide receptor). Morphine is, thus, the starting point for an analysis of the role of opioid receptors in cSNC.

Rowan and Flaherty (1987) first reported that the pretrial systemic administration of morphine (4 and 8 mg/kg), whether before trial 11 or trial 12, attenuated cSNC without completely eliminating the effect. These doses had no detectable effect on consummatory behavior in rats exposed only to 4% sucrose (unshifted controls), but increased consummatory behavior in rats exposed to the 32-to-4% sucrose downshift. A higher dose of morphine (16 mg/kg) also disrupted the consummatory behavior of unshifted controls, thus making it difficult to interpret the effects of morphine on the consummatory behavior of downshifted rats. Rowan and Flaherty also reported that naloxone, a nonselective OR antagonist with greater affinity for the MOR, failed to disrupt cSNC (0.25, 0.5, and 1.0 mg/kg) when administered by itself. However, naloxone (0.5 mg/kg) eliminated the attenuating effects of morphine (4 mg/kg) when both were coadministered.

ORs are Engaged in cSNC

There were at least two potential problems with the naloxone data reported by Rowan and Flaherty (1987). First, because naloxone is expected to enhance cSNC, a 32-to-4% sucrose downshift could leave little room to detect further suppression of consummatory behavior, especially on trial 11 (i.e., a floor effect). Second, whereas the dose (0.5 mg/kg) used was sufficient to abolish the effects of morphine on cSNC, it may have been insufficient to have effects on its own. With these caveats in mind, Pellegrini, Wood, Daniel, and Papini (2005) exposed rats to a 32-to-6% sucrose downshift while administering a 2 mg/kg dose prior to trials 11 and 12. The treatment successfully enhanced cSNC. As shown in Figure 3, there was evidence of cSNC in both the saline and naloxone pairs of downshifted vs. unshifted groups, but whereas naloxone had no effect on unshifted rats, it significantly reduced consummatory behavior in downshifted rats. Moreover, whereas the cSNC effect (i.e., the difference between downshifted vs. unshifted groups) lasted two trials in the saline comparison (trials 11-12), it lasted at least 5 trials in the naloxone comparison (trials 11-15).

The enhancing effects of naloxone on cSNC were not restricted to these particular conditions. In a second experiment, Pellegrini et al. (2005) reported naloxone-induced consummatory suppression after the more conventional 32-to-4% sucrose downshift (2 mg/kg). This naloxone effect is not an automatic consequence of a downshift experience because a relatively mild reduction in sucrose concentration does not lead to enhanced consummatory suppression. For example, naloxone leads to significant suppression after 32-to-6% or 32-to-12% sucrose downshifts, but not after 16-to-3% or 16-to-6% sucrose downshifts

(Daniel, Ortega, & Papini, 2009). Thus, opioid blockage is hypothesized to enhance the frustrative response to incentive loss, which in turn augments the cSNC effect.

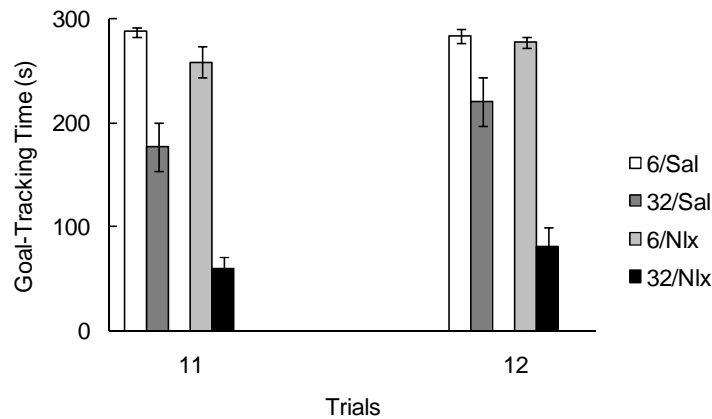


Figure 3. Effects of naloxone on cSNC (Pellegrini et al., 2005). Naloxone is a nonselective opioid receptor antagonist with greater affinity for the MOR. Rats were exposed for 10 trials to either 32% or 6% sucrose. On trial 11, downshifted animals had access to 6% sucrose (rather than the usual 32% sucrose), whereas unshifted controls continue to access the same 6% solution of previous trials. Naloxone (2 mg/kg, ip) was administered 15 min before trials 11 and 12 (shown in this figure). In both trials, there was significantly more suppression of goal-tracking times in the group treated with naloxone (Nlx) than in the saline (Sal) groups. Goal-tracking time is the cumulative time in contact with the sipper tube during the trial.

OR Blockage Alters the Detection of the Incentive Downshift

Detecting a downshift in sucrose concentration is not a purely perceptual problem. The cSNC effect requires a comparison between the sweetness of the current (postshift) solution and the reactivated memory of a previously experienced (preshift) solution. Papini and Pellegrini (2006) showed that, within some limits, equal ratios of postshift/preshift sucrose concentrations yield similar levels of consummatory suppression. For example, a 32-to-4% sucrose downshift leads to similar goal-tracking times as a 16-to-2% sucrose downshift; in both cases, the downshift involves an 8-to-1 downshift ratio. In addition, the smaller the ratio, the lesser the consummatory suppression (i.e., an 8-to-1 ratio induces more suppression than a 4-to-1 ratio). This ratio constancy is analogous to Weber's law as applied to comparisons between sensory inputs and it applies to a variety of incentive downshift situations in addition to cSNC (Pellegrini & Papini, 2007; Pellegrini, Lopez-Seal, & Papini, 2008).

Recent data suggest that OR blockage alters the downshift detection rule from a ratio to an absolute difference rule (Daniel et al., 2009). Saline treated rats exhibited similar suppression of consummatory behavior when given a 16-to-6% vs. 32-to-12% sucrose downshift (post/pre ratio = 0.38), or 16-to-3% vs. 32-to-6% sucrose downshift (post/pre ratio = 0.19). Interestingly, rats treated with naloxone (2 mg/kg) exhibited a level of consummatory suppression on trial 11 that was more

in synchrony with the absolute difference between the pre- and postshift sucrose concentrations, rather than with their ratio. The consummatory behavior of these animals on the first downshift trial (trial 11) yielded a coefficient of determination $r^2 = 0.77$, whereas the same data for the saline controls yielded $r^2 = 0.42$. The difference indicates that a linear function relating consummatory behavior to the absolute difference in concentrations provides a better fit for naloxone-treated animals than for saline-treated animals. Linearity is distorted in saline animals because of ratio constancy. These results suggest that OR blockage distorts the comparison between the current solution and the reactivated memory of the preshift solution, biasing it in the direction of the absolute difference between the two solutions, rather than of their ratio.

DORs Selectively Modulate cSNC Onset

Flaherty (1996) reviewed data showing that benzodiazepine anxiolytics displayed trial selectivity, reducing contrast when administered before trial 12, but not before trial 11. Based on such evidence, Flaherty suggested that recovery from cSNC involved a conflict between the rejection of the downshifted solution and the need to consume sucrose given that animals are usually food deprived in these experiments. In fact, recovery from cSNC is retarded when rats are not food deprived, suggesting that satiety reduces the approach component of the conflict (Dachowski & Brazier, 1991). But none of the extensive series of pharmacological experiments summarized by Flaherty (1996) proved to selectively modulate cSNC on trial 11, during the very first exposure to the downshifted solution.

To test for selective modulation of cSNC on trial 11 vs. 12, Wood, Daniel, and Papini (2005) gave three downshifted-unshifted pairs of groups injections before each of these two trials. One pair received DPDPE (24 $\mu\text{g}/\text{kg}$) before trial 11, but the vehicle before trial 12; a second pair received the vehicle before trial 11, but DPDPE before trial 12; and the third pair of groups received the vehicle before both trials. DPDPE is a selective DOR agonist and, thus, it was expected to reduce cSNC much as morphine did in prior experiments (Rowan & Flaherty, 1987). Surprisingly, however, the attenuating effect of DPDPE was restricted to trial 11, as shown in Figure 4. Although DPDPE-treated downshifted rats showed somewhat lower consummatory behavior on trial 11, the difference with DPDPE-treated unshifted rats was not significant. In addition, DPDPE had no effect when administered before trial 12 or in unshifted controls. Another experiment (Pellegrini et al., 2005) showed that administration of the DOR antagonist naltrindole (1 mg/kg) before trials 11 and 12 enhanced cSNC on trial 11, but had no effect on trial 12. Based on these results, it was hypothesized that DORs play an important and selective role in the onset of the cSNC effect, modulating the intensity of primary frustration, that is, an unconditioned state peaking immediately after a surprising incentive downshift and hypothesized to play a major role in consummatory suppression during trial 11.

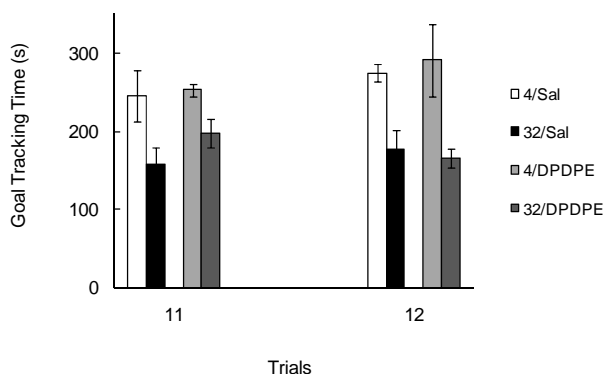


Figure 4. Effects of DPDPE on cSNC (Wood et al., 2005). DPDPE is a selective DOR agonist. Groups of rats received DPDPE (24 μ g/kg, ip) administration either before trial 11 or before trial 12. Whereas DPDPE significantly reduced cSNC when administered before trial 11, it had no effect when administered before trial 12. A control group received saline (Sal) administration before both trials.

KORs Selectively Modulate Recovery from cSNC

Another recent series of experiments with the KOR agonist U50,488H provided additional evidence for trial selectivity (Wood, Norris, Daniel, & Papini, 2008). In this case, U50,488H administered before trial 11 had no detectable effect on cSNC, but before trial 12 led to either attenuation (1 mg/kg) or enhancement (3 and 10 mg/kg) of cSNC. Subsequent experiments showed that the attenuating effect of the 1 mg/kg dose failed to occur when U50,488H was administered immediately after trial 11, whereas the enhancing effect of the 3 mg/kg occurred also when it was administered immediately after trial 11. Additional data suggested that the enhancing effect of the 3 mg/kg dose was probably due to the development of a conditioned taste aversion (CTA), as animals given 4% sucrose (i.e., without a downshift) and injected immediately after the trial exhibited less consummatory behavior than animals injected 3 h after the trial (i.e., paired vs. unpaired sucrose-U50,488H trials). Therefore, the enhancing effect of the high dose of U50,488H was tentatively dismissed as due to CTA, whereas the effect of a low dose of U50,488H on cSNC was hypothesized to be similar to that of benzodiazepine anxiolytics in that it is selective for trial 12. Thus, KORs are hypothesized to modulate the intensity of secondary frustration, that is, a conditioned state induced by anticipated frustration, assumed to play a major role in consummatory suppression during trial 12.

What is the Function of ORs in cSNC?

The enhancing effects of naloxone on cSNC described above can be attributed to at least four mechanisms. First, OR blockage may modulate the

downshift experience, either amplifying the aversive consequences of surprising reward reductions (Papini & Dudley, 1997), reducing the incentive value of the downshifted solution (Norris, Perez-Acosta, Ortega, & Papini, in press), or a combination of both effects. Second, naloxone could exert this effect by affecting the detection of the downshift, as shown above. Additional data are needed to evaluate these possibilities.

Third, naloxone may induce a CTA analogous to that observed with U50,488H. The logic underlying this CTA hypothesis is based on the notion that when 4% sucrose is paired with the aversive state induced by a drug on trial 11, downshifted rats have never tasted 4% sucrose before, whereas unshifted controls have experienced 10 previous trials with 4% sucrose. Thus, since CTA occurs more readily with novel flavors (i.e., latent inhibition; Cannon, Best, & Batson, 1983), downshifted rats would be more likely than unshifted rats to develop an aversion to the 4% sucrose, resulting in an apparent enhancement of the cSNC effect. Despite the potential for CTA, administration of naloxone (2 and 10 mg/kg) immediately after the first experience with 4% sucrose (i.e., in the absence of a downshift experience) failed to support CTA relative to groups receiving either unpaired or backward arrangements between sucrose and naloxone. It should be noted that no information is available on the ability of naltrindole, which also enhances cSNC (see above), to support CTA.

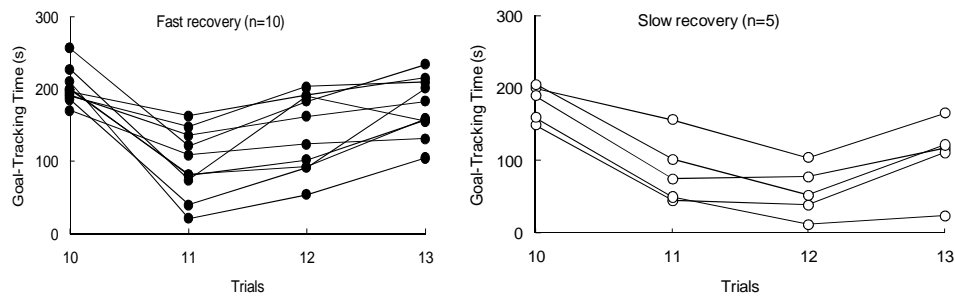
Fourth, OR blockage could enhance cSNC by strengthening the aversive memory of the incentive downshift event first experienced on trial 11. Posttraining naloxone administration is known to enhance the consolidation of fear conditioning (see McGaugh & Roozendaal, 2008). However, the same doses of naloxone (2 mg/kg), naltrindole (1 mg/kg), and DPDPE (24 µg/kg) that were effective when administered before trials 11 and/or 12, had no effect on recovery when administered immediately after trial 11 (Daniel et al., 2009). Therefore, it is hypothesized that ORs are not involved in the encoding of secondary frustration in the cSNC situation.

ORs and Individual Differences in Recovery from cSNC

cSNC is a robust phenomenon, but there are notable differences in the length of the effect across experiments run under the same nominal conditions. Recovery from incentive downshift may take between 1 and 6 postshift trials. In addition, there are substantial individual differences in both the extent of the initial suppression (trial 11) and the speed of the subsequent recovery (trial 12 and beyond), as shown in Figure 5a. Because ORs are implicated in both aspects of the cSNC effect, Pellegrini et al. (2005) hypothesized that individual differences in postshift performance reflect the efficiency of endogenous opioid ligands to their receptors. Rats and humans express several OR isoforms that bind with different effectiveness and play a role in drug addiction (e.g., Ikeda et al., 2005). Thus, an experiment was designed to test the hypothesis that rats that expressed fast vs. slow recovery from cSNC (as measured in terms of the performance on trials 11 and 12) exhibit differential sensitivity to OR blockage in an activity situation.

Activity was measured in a narrow, dark, and walled box designed to minimize anxiety-like responses that rats often exhibit in open, lighted spaces (Pawlak, Ho, & Schwarting, 2008). Groups matched for performance on trial 11 (i.e., equal initial suppression) that exhibited either fast or slow recovery of goal-tracking times on trial 12 received naloxone (2 mg/kg) treatment immediately before a 15-min activity session. As predicted, whereas naloxone had no effect on activity for fast-recovery rats, it significantly reduced activity late in the session for slow-recovery rats (Figure 5b). These results were interpreted as providing support for the hypothesis that the animal's ability to cope with an experience of incentive downshift is directly related to OR effectiveness.

(a) Individual differences in recovery from incentive downshift.



(b) Effects of naloxone on activity in fast-recovery vs. slow-recovery rats.

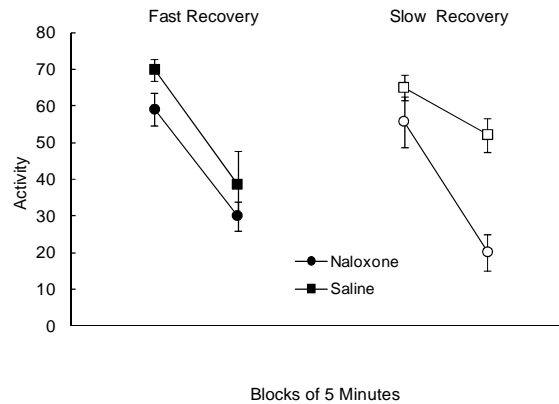


Figure 5. (a) Unpublished data showing individual performance during the last preshift trial (trial 10) of access to 32% sucrose and the initial three postshift trials (trials 11-13) of access to 4% sucrose. Animals differ in terms of their performance on trial 12 relative to trial 11: scores go up for fast recovery rats, but stay the same or go down for slow recovery rats. (b) Effects of naloxone (2 mg/kg, ip) on activity in groups of rats that exhibited either fast or slow recovery during a 32-to-4% sucrose downshift (Pellegrini et al., 2005).

Evolution of the Opioid System

Using Figure 2 as a guide, one could argue that despite its complexity, SNC is beginning to be understood at the behavioral and neurochemical levels. Whereas nothing much can be said about the neural circuit and the cell-molecular processes underlying SNC, the behavioral and neurochemical research described in this article offers a general guide to generate some hypotheses about the possible evolutionary history of the mechanisms underlying SNC. It is useful to start by making explicit two assumptions: (1) that the behavioral differences illustrated in Figure 1 reflect a divergence in learning mechanisms underlying adjustments to incentive downshifts, rather than the effect of some contextual variable unrelated to learning (e.g., perceptual, motivational, or motor differences across species; Bitterman, 1975); and (2) that the set of mechanisms underlying SNC evolved in Mesozoic mammals (or their ancestors) by means of co-option of brain mechanisms originally involved in fear conditioning (Papini, 2003, 2006). Some set of fear conditioning mechanisms appears to be general to most, if not all, vertebrates, as shown by research on avoidance learning in teleost fish (*Carassius auratus*; Portavella, Salas, Vargas, & Papini, 2003; Portavella, Torres, Salas, & Papini, 2004). Based on these assumptions and on the results reviewed above, it is hypothesized that evolutionary changes in the functions of ORs made SNC possible in mammals.

The evolution of the opioid system is beginning to be understood by recent work with a variety of vertebrates, thanks to the involvement of ORs in nociception (Sneddon, 2004). The four recognized ORs (delta, mu, kappa, and ORL), whose genes share a similar sequential structure, have been identified in mammals, birds, reptiles, amphibians, and teleost fish, but not in chondrychthyes (sharks), cephalochordates (lancelets), urochordates (tunicates), or arthropods (fruit flies; Dreborg et al., 2008; Stevens, 2009). There is some indication that endogenous ligands may bind with less specificity in nonmammalian ORs than in their mammalian homologues (Stevens, Brasel, & Mohan, 2007), but the status of this claim is uncertain. ORs may be equally selective across vertebrates, but their selectivity may relate to different endogenous ligands (Dreborg et al., 2008). Studies using selective radioligands for the DOR, MOR, and KOR in the amphibian *Rana pipiens* show that binding to these ORs is just as specific as it is for mammals (Newman, Sands, Wallace, & Stevens, 2002). Interestingly, although the amino acid sequences in these ORs are very similar both across species and within species, nonmammalian ORs are more similar to each other than mammalian ORs (Stevens et al., 2007). This suggests greater divergence in OR structure in the mammalian lineage compared to nonmammalian vertebrates. The extent to which this divergence allowed the mammalian opioid system to extend its influence to situations involving incentive loss remains to be determined. However, a tentative evolutionary hypothesis is presented in Figure 6.

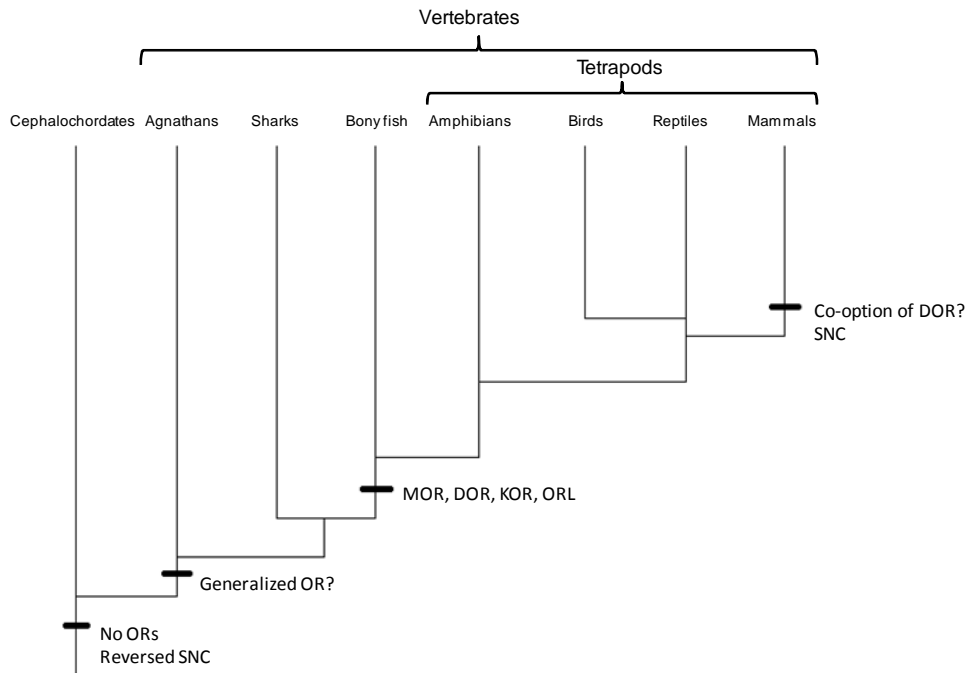


Figure 6. A phylogenetic tree showing the main vertebrate lineages and the hypothesized events in the evolution of opioid receptors. The four described opioid receptors, MOR, DOR, KOR, and ORL, have been found in bony fish and tetrapods. Because these proteins are characterized by a high degree of sequence similarity, they have been hypothesized to have evolved in two events of gene duplication from a generalized ancestral protein. Here it is also hypothesized that the DOR underwent co-option from playing a role in the pain-fear domain to playing a role in the onset of incentive contrast situations.

The evolutionary hypothesis based on the functional co-option of fear conditioning mechanisms into those subserving adjustment to incentive loss (Papini, 2003) requires some specificity as to the nature of those functions for the opioid system. In relation to fear conditioning, the opioid system has been suggested to play a role in modulating (1) the intensity of the shock-induced pain and signal-induced fear (Fanselow & Bolles, 1979), (2) the magnitude of the error-correction mechanism involved in fear acquisition and extinction (McNally, in press), and (3) the consolidation of the fear memory (McGaugh & Roozendaal, 2008). As reviewed in this article, the functional role of the opioid system suggested by research on incentive downshift would be consistent with (1), uncertain about (2), and inconsistent with (3). But the task of comparing the function of the same neurochemical system on different behavioral functions is rather complex. Consider the opposite effects of opioid blockage on extinction. Naloxone treatment retards fear extinction (McNally & Westbrook, 2003), but it facilitates appetitive extinction (Norris et al., in press). Different behavioral effects may reflect either the same or different opioid function. For example, if opioid

blockage enhances aversive emotional states, then a signal for pain should cause more intense fear (thus retarding fear extinction) and more intense frustration (thus enhancing appetitive extinction). On the other hand, opposite effects may reflect different functions. For example, opioid blockage may interfere with error correction in fear extinction (McNally, 2009), but have a different function in the case of appetitive extinction. Clearly, the evidence is presently insufficient to determine whether evolutionary changes in the opioid system can be meaningfully related to opioid function in the pain-fear and frustration domains.

Conclusions

Based on a level's view of the mechanisms underlying SNC, the research described here was designed to build on the extensive work on the neurochemical basis of cSNC published by Flaherty and collaborators. Systemic administration of opioid peptides was selected as an initial manipulation because a series of experiments indicated that morphine reduced the initial impact of incentive downshift in the consummatory situation (Rowan & Flaherty, 1987). Systemic drug administration is a relatively practical approach to determine some general effects and identify ORs involved in cSNC, but it has at least two disadvantages related to the widespread distribution of ORs in the mammalian brain. First, it does not tell us where in the brain a given opioid peptide is causing the observed behavioral effects. Second, the same compound acting in different brain sites may affect behavior in opposite ways, thus obscuring the outcome of some experiments. Nonetheless, systemic administration provides a rough roadmap to target specific brain sites. Lesion studies implicate several brain nuclei in the development of cSNC, including the parabrachial nucleus (Grigson, Spector, & Norgren, 1994), gustatory thalamus (Sastre & Reilly, 2006), medial amygdala (Becker, Jarvis, Wagner, & Flaherty, 1984), and medial prefrontal cortex (Pecoraro, De Jong, Ginsberg, & Dallman, 2008). A study using c-Fos-like immunoreactivity identified the medial amygdala and a variety of brain areas activated selectively on trial 11, during initial exposure to incentive downshift (Pecoraro & Dallman, 2005). Such studies provide a guide to explore the role of ORs in specific brain locations on cSNC onset using microinjection procedures (e.g., Liao & Chuang, 2003).

Once the minimum circuitry for cSNC is known, it would be possible to approach the homologous brain areas in other vertebrates to determine similarities and differences across species. Potential nonmammalian models include the goldfish (*Carassius auratus*), terrestrial toad (*Bufo arenarum*), and pigeon (*Columba livia*). These species have been extensively studied in incentive downshift situations and the behavioral effects are strikingly different from those observed in mammals. Recent research with pigeons shows, for example, that their adjustment to a downshift in incentive is regulated primarily by the magnitude of the preshift incentive, rather than the ratio of post-to-preshift magnitudes, as described above for rats (Pellegrini et al., 2008). Such systematic study of key model species will open the way to a deeper understanding of the evolution of learning mechanisms in vertebrates.

References

- Agranoff, B. W., Davis, R. E., & Brink, J. J. (1966). Chemical studies on memoryfixation in goldfish. *Brain Research, 1*, 303-309.
- Amsel, A. (1992). *Frustration theory*. Cambridge, UK: Cambridge University Press.
- Becker, H. C., Jarvis, M., Wagner, G., & Flaherty, D. F. (1984). Medial and lateral amygdala lesions differentially influence contrast with sucrose solutions. *Physiology & Behavior, 33*, 707-712.
- Bentosela, M., Jakovcevic, A., Elgier, A. M., Mustaca, A. E., & Papini, M. R. (2009). Incentive contrast in domestic dogs (*Canis familiaris*). *Journal of Comparative Psychology, 123*, 125-130.
- Bitterman, M. E. (1975). Comparative analysis of learning. *Science, 188*, 699-709.
- Bitterman, M. E. (1976). Incentive contrast in honey bees. *Science, 192*, 380-382.
- Brownstein, M. J. (1993). A brief history of opiates, opioid peptides, and opioid receptors. *Proceedings of the National Academy of Sciences USA, 90*, 5391-5393.
- Cannon, D. S., Best, M. R., & Batson, J. D. (1983). Taste familiarity and apomorphine-induced taste aversion. *Behavior Research & Therapy, 21*, 669-673.
- Couvillon, P. A., & Bitterman, M. E. (1984). The overlearning-extinction effect and successive negative contrast in honeybees (*Apis mellifera*). *Journal of Comparative Psychology, 98*, 100-109.
- Dachowski, L., & Brazier, M. M. (1991). Consummatory incentive contrast: Experimental design relationships and deprivation effects. In L. Dachowski & C. F. Flaherty (Eds.), *Current topics in animal learning* (pp. 245-270). Hillsdale, NJ: Erlbaum.
- Daniel, A. M., Ortega, L. A., & Papini, M. R. (2009). Role of the opioid system in incentive downshift situations. *Neurobiology of Learning and Memory, 92*, 439-450.
- Dreborg, S., Sundström, G., Larsson, T. A., & Larhammar, D. (2008). Evolution of vertebrate opioid receptors. *Proceedings of the National Academy of Sciences USA, 105*, 15487-15492.
- Elliott, M. H. (1928). The effect of change of reward on the maze performance of rats. *University of California Publications in Psychology, 4*, 19-30.
- Fanselow, M. S., & Bolles, R. C. (1979). Naloxone and shock-elicited freezing in the rat. *Journal of Comparative and Physiological Psychology, 93*, 736-744.
- Flaherty, C. F. (1996). *Incentive relativity*. Cambridge, UK: Cambridge University Press.
- Freidin, E., Cuello, M. I., & Kacelnik, A. (2009). Successive negative contrast in a bird: Starlings' behavior after unpredictable negative changes in food quality. *Animal Behaviour, 77*, 857-865.
- Gray, J. A. (1987). *The psychology of fear and stress*. Cambridge: Cambridge University Press.
- Grigson, P. S., Spector, A. C., & Norgren, R. (1994). Lesions of the pontine parabrachial nuclei eliminate successive negative contrast effects in rats. *Behavioral Neuroscience, 108*, 714-723.
- Ikeda, K., Ide, S., Han, W., Hayashida, M., Uhl, G. R., & Sora, I. (2005). How individual sensitivity to opiates can be predicted by gene analyses. *Trends in Pharmacological Sciences, 26*, 311-317.
- Kandel, E., & Abel, T. (1995). Neuropeptides, adenylyl cyclase, and memory storage. *Science, 268*, 825-826.
- Lashley, K. S. (1929). *Brain mechanisms and intelligence*. Chicago, IL: Chicago University Press.

- Lowes, G., & Bitterman, M. E. (1967). Reward and learning in the goldfish. *Science*, *157*, 455-457.
- Liao, R.-M., & Chuang, F.-C. (2003). Differential effects of diazepam infused into the amygdala and hippocampus on negative contrast. *Pharmacology Biochemistry and Behavior*, *74*, 953-960.
- Mansour, A., Fox, C. A., Akil, H., & Watson, S. J. (1995). Opioid-receptor mRNA expression in the rat CNS: Anatomical and functional implications. *Trends in Neuroscience*, *18*, 22-29.
- McGaugh, J. L., & Roozendaal, B. (2008). Memory modulation. In J. H. Byrne (Ed.), *Learning and memory: A comprehensive reference, Vol. 3* (pp. 521-553). New York: Elsevier.
- McNally, G. P. (2009). The roles of endogenous opioids in fear learning. *International Journal of Comparative Psychology*, *22*(3), 154-170.
- McNally, G. P., & Akil, H. (2002). Opioid peptides and their receptors: Overview and function in pain modulation. In K. Davis, D. Charney, J. T. Coyle, & C. Nemeroff (Eds). *Neuropsychopharmacology: Fifth generation of progress* (pp. 35-46). New York: Lippincott, Williams, & Wilkins.
- McNally, G. P., & Westbrook, R. F. (2003). Opioid receptors regulate the extinction of Pavlovian fear conditioning. *Behavioral Neuroscience*, *117*, 1292-1301.
- Newman, L. C., Sands, S. S., Wallace, D. R., & Stevens, C. W. (2002). Characterization of m, k, and d opioid binding in amphibian whole brain tissue homogenates. *Journal of Pharmacology & Experimental Therapeutics*, *301*, 364-370.
- Norris, J. N., Perez-Acosta, A. M., Ortega, L. A., & Papini, M. R. (in press). Naloxone facilitates appetitive extinction and eliminates escape from frustration. *Pharmacology, Biochemistry & Behavior*.
- Papini, M. R. (1997). Role of reinforcement in spaced-trial operant learning in pigeons (*Columba livia*). *Journal of Comparative Psychology*, *111*, 275-285.
- Papini, M. R. (2002). Pattern and process in the evolution of learning. *Psychological Review*, *109*, 186-201.
- Papini, M. R. (2003). Comparative psychology of surprising nonreward. *Brain, Behavior and Evolution*, *62*, 83-95.
- Papini, M. R. (2006). Role of surprising nonreward in associative learning. *Japanese Journal of Animal Psychology*, *56*, 35-54.
- Papini, M. R. (2008). *Comparative psychology: Evolution and development of behavior*. New York: Psychology Press.
- Papini, M. R., & Dudley, R. T. (1997). Consequences of surprising reward omissions. *Review of General Psychology*, *1*, 175-197.
- Papini, M. R., & Ishida, M. (1994). Role of magnitude of reinforcement in spaced-trial instrumental learning in turtles (*Geoclemys reevesii*). *Quarterly Journal of Experimental Psychology*, *47B*, 1-13.
- Papini, M. R., Muzio, R. N., & Segura, E. T. (1995). Instrumental learning in toads (*Bufo arenarum*): Reinforcer magnitude and the medial pallium. *Brain, Behavior and Evolution*, *46*, 61-71.
- Papini, M. R., & Pellegrini, S. (2006). Scaling relative incentive value in consummatory behavior. *Learning & Motivation*, *37*, 357-378.
- Pavlov, I. P. (1927). *Conditioned reflexes*. Oxford, UK: Oxford University Press.
- Pawlak, C. R., Ho, Y.-J., & Schwarting, R. K. W. (2008). Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. *Neuroscience & Biobehavioral Reviews*, *32*, 1544-1568.

- Pecoraro, N., & Dallman, M. F. (2005). c-Fos after incentive shifts: Expectancy, incredulity, and recovery. *Behavioral Neuroscience*, *119*, 366-387.
- Pecoraro, N., De Jong, H., Ginsberg, A. B., & Dallman, M. F. (2008). Lesions of the medial prefrontal cortex enhance the early phase of psychogenic fever to unexpected sucrose concentration reductions, promote recovery from negative contrast, and enhance spontaneous recovery of sucrose-entrained anticipatory activity. *Neuroscience*, *153*, 901-917.
- Pellegrini, S., Lopez-Seal, M. F., & Papini, M. R. (2008). Scaling relative incentive value: Different adjustments to incentive downshift in pigeons and rats. *Behavioural Processes*, *79*, 182-188.
- Pellegrini, S., & Papini, M. R. (2007). Scaling relative incentive value in anticipatory behavior. *Learning & Motivation*, *38*, 128-154.
- Pellegrini, S., Wood, M., Daniel, A., & Papini, M. R. (2005). Opioid receptors modulate recovery from consummatory successive negative contrast. *Behavioural Brain Research*, *164*, 239-249.
- Portavella, M., Salas, C., Vargas, J. P., & Papini, M. R. (2003). Involvement of the telencephalon in spaced-trial avoidance learning in the goldfish (*Carassius auratus*). *Physiology & Behavior*, *80*, 49-56.
- Portavella, M., Torres, B., Salas, C., & Papini, M. R. (2004). Lesions of the medial pallidum, but not of the lateral pallidum, disrupt spaced-trial avoidance learning in goldfish (*Carassius auratus*). *Neuroscience Letters*, *24*, 2335-2342.
- Potts, A., & Bitterman, M. E. (1967). Puromycin and retention in the goldfish. *Science*, *158*, 1594-1596.
- Rowan, G. A., & Flaherty, C. F. (1987). The effects of morphine in the consummatory contrast paradigm. *Psychopharmacology*, *93*, 51-58.
- Sastre, A., & Reilly, S. (2006). Excitotoxic lesions of the gustatory thalamus eliminate consummatory but not instrumental successive negative contrast in rats. *Behavioural Brain Research*, *170*, 34-40.
- Sim-Selley, L. J., Vogt, L. J., Childers, S. R., & Vogt, B. A. (2003). Distribution of ORL-1 receptor binding and receptor-activated G-proteins in rat forebrain and their experimental localization in anterior cingulate cortex. *Neuropharmacology*, *45*, 220-230.
- Sneddon, L. U. (2004). Evolution of nociception in vertebrates: Comparative analysis of lower vertebrates. *Brain Research Reviews*, *46*, 123-130.
- Stevens, C. W. (2009). The evolution of vertebrate opioid receptors. *Frontiers in Bioscience*, *14*, 1247-1269.
- Stevens, C. W., Brasel, C. M., & Mohan, S. (2007). Cloning and bioinformatics of amphibian mu, delta, kappa, and nociceptin opioid receptors expressed in brain tissue: Evidence for opioid receptor divergence in mammals. *Neuroscience Letters*, *419*, 189-194.
- Thorndike, E. L. (1911). *Animal intelligence*. New York: Lemcke & Buechner.
- Wagner, A. R. (1969). Frustrative nonreward: A variety of punishment. In B. A. Campbell & R. M. Church (Eds.), *Punishment and aversive behavior* (pp. 157-181). New York: Appleton-Century-Crofts.
- Waldron, F. A., Wiegmann, D. D., & Wiegmann, D. A. (2005). Negative incentive contrast induces economic choice behavior by bumble bees. *International Journal of Comparative Psychology*, *18*, 358-371.
- Wood, M. D., Daniel, A. M., & Papini, M. R. (2005). Selective effects of the δ opioid receptor agonist DPDPE on consummatory successive negative contrast. *Behavioral Neuroscience*, *119*, 446-454.

Wood, M. D., Norris, J. N., Daniel, A. M., & Papini, M. R. (2008). Trial-selective effects of U50,488H, a κ -opioid receptor agonist, on consummatory successive negative contrast. *Behavioural Brain Sciences*, *193*, 28-36.

Original received February 26, 2009.

Revision received July 28, 2009.

Accepted July 31, 2009.