A perspective on the Contributions of Kent Berridge and of Suzanne Higgs & Steven J. Cooper

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Abstract

Kent Berridge has been instrumental to understanding rat taste and ingestive behavior. Using the taste reactivity paradigm developed under Grill and Norgren, Dr. Berridge has contributed the action of benzodiazepines in the parabrachial nucleus of the pons to increase food intake by potentiating the hedonic impact of food. He has further addressed the role of opioids in the nucleus of accumbens in a similar action on palatability. In addition, Berridge’s lab has also contributed to ruling out an effect of dopaminergic, serotonergic and the lateral hypothalamus in palatability. Although his continued research often focuses on addiction and motivation, his earlier work on ingestive behavior has wide implications and applications. Likewise, Suzanne Higgs and Steven J. Cooper contributed to an understanding of the pharmacology of taste. Through both microstructural licking analyses and application of the taste reactivity paradigm, Higgs and Cooper helped locate the action of benzodiazepines to increase palatability. Furthermore they contributed to an understanding of the importance of endogenous opioids in benzodiazepine-induced enhancement of palatability.
A perspective on the Contributions of Kent C. Berridge and of Suzanne Higgs & Steven J. Cooper

Introduction

Compared to other sensory systems, not much is known about taste. Research into taste and ingestive behavior has lately been fueled by concern over the obesity epidemic, but for previous generations of neuroscientists, other fields drew more focus. Significant contributors to knowledge of taste in the rat model are Kent Berridge and the collaboration of Suzanne Higgs and Steven Cooper. An early pharmacological tool for investigating the neurochemical mechanisms of taste was identified in the GABA agonist family, benzodiazepines. Berridge and Higgs and Cooper used benzodiazepines the examine GABA influences on taste processes related to ingestive behavior.

Kent C. Berridge received a Bachelor’s of Science at the University of California at Davis in 1979. At the University of Pennsylvania, under the mentorship of Harvey J. Grill, Berridge earned a Masters in 1980 and a Ph.D. in 1983. Currently the James Olds Collegiate Professor of Psychology and Neuroscience at the University of Michigan (Ann Arbor, USA), he has received numerous awards and honors including a Guggenheim Fellowship and a Distinguished Early Scientific Career Award from the American Psychological Association. Much of his current research focuses on addiction, pleasure, and the difference between wanting and liking (Berridge, n.d.). In exploring this last interest, Dr. Berridge has contributed much to the field of rat taste and ingestive behaviors.

Steven J. Cooper received a Ph.D. in Psychopharmacology at the University of London in 1970. He taught at the University of Birmingham and the University of Durham before settling at the University of Liverpool where he oversaw the transition of the department of psychology into a freestanding school. Over a lifetime of research in the psychopharmacology of appetite, he
received numerous awards and honors. Shortly before his death in 2007, he helped found the Liverpool Obesity Research Network (Halford, n.d.).

Appointed the title of reader in psychobiology at the University of Birmingham, Suzanne Higgs completed her Ph.D. in psychopharmacology at the University of Durham under Steven Cooper (Higgs, n.d.). Their work together was devoted to pharmacological research on appetite. By using psychoactive drugs as pharmacological tools, they were able to investigate the neurochemical mechanisms involved in ingestive behavior. Applying analyses of lick behavior, the duo was able to investigate drug effects on palatability, beyond mere intake measures. Their work, then and since, has focused on the role of benzodiazepines, opioids, and dopamine in palatability.

This paper will review the contributions of Kent Berridge and of Steven Cooper and Suzanne Higgs to the field of taste research. In addition to their work with benzodiazepines, pharmacological studies examining the role of dopamine and opioids are also addressed. Overall, Berridge and Cooper and Higgs have helped build a solid foundation of knowledge of taste and ingestion upon which neuroscientists can build.

Kent C. Berridge

As a young graduate student in 1978, Berridge helped to refine the taste reactivity paradigm in the lab of Harvey J. Grill (Grill & Norgren, 1978). The taste reactivity paradigm functions as a means to measure taste affect in rats. By infusing tastant solutions through chronic intraoral cannulae implanted lateral to the first molars, taste reactivity could be measured without interfering with normal ingestion or interference from the gut. Counting stereotypical aversive, ingestive, and negative reactions of the face, mouth and paws generates a response profile to a
tastant. Actions rated as strongly ingestive include paw licking, nonrhythmic lateral tongue protrusions, and rhythmic tongue protrusions along the midline. Strongly aversive actions include gaping of the mouth, rubbing of the chin on the floor, face washing, flailing of the forelimbs, rapid headshaking, and paw treading. Passive drip and rhythmic mouth movement without tongue protrusion are rated as neutral. Together these actions constitute the taste affect profile. Taste affect has been shown to correspond to intake and to be modulated by the same variables, such as hunger and caloric satiety, which affect palatability in humans.

In addition to identifying the similarities of variables modulating taste affect between rats and humans, Berridge also used the taste reactivity paradigm to identify the possibility of a two dimensional aspect of palatability in rats (Berridge et al., 1981; Berridge et al., 1984; Berridge & Grill, 1983). It was found that one tastant could elicit both aversive and ingestive reactions (Berridge & Grill, 1983). For example, high concentrations of sucrose were shown to induce rapid vacillation between strong positive ingestive reactions and weak or mild aversive reactions. Such a rapid alternation of consummatory responses between positive and aversive indicated the simultaneous activation of two dimensions of palatability, one for hedonic impact and one for aversive. If palatability of a tastant were a point on a single spectrum between good and bad, then the tastant would more likely elicit neutral responses. This observation was ultimately helpful in applying the taste reactivity paradigm, as a richer taste profile could be built on just one tastant. Sucrose was used for sweet, normally inducing ingestive responses. HCl normally elicits ingestive and aversive reactions mixed. And quinine is an aversive tastant. But a bittersweet mix of sucrose and quinine is often sufficient to find an effect of experimental conditions on palatability.
One of the areas of ingestive behavior to which Kent Berridge greatly contributed regarded the role benzodiazepines play in inducing hyperphagia. Benzodiazepines are a class of GABA agonist drugs used for their anxiolytics effects and often to treat alcoholism (Sigel & Steinmann, 2012). GABAA receptors mediate neuronal inhibition, making them responsible for a majority of inhibitory action in the mammalian brain. Benzodiazepines do not act as a direct agonist. By binding to GABAA receptors, the drugs potentiate the effect of endogenously released GABA by allowing the channel to stay open longer when both benzodiazepines and GABA are present.

An early noted effect of benzodiazepines was the increase in food intake by rats (Randall & Kappell, 1961). This hyperphagia was thought to be a result of reduced anxiety rather than of any direct biological action on the neural mechanisms of ingestion (Britton et al., 1981; Vogel, Beer & Clody, 1971). Wise was the first to suggest that benzodiazepines may act directly on a feeding mechanism, following observations that, rather than a result of metabolic degradation of the drug, termination of feeding in rats treated with diazepam was an effect of feedback from the gut (Wise & Dawson, 1974). In the early 1980’s Cooper and McClelland noted that benzodiazepines selectively increased intake of preferred foods, thereafter developing a hypothesis that benzodiazepines act by increasing the palatability of food (Cooper & McClelland, 1980). Pharmacological testing of anxiolytics used increased ingestion as a measure of drug efficacy (Vogel, Beer & Clody, 1971). Therefore it was crucial to resolve the question regarding the drugs’ method of action. This question could not be resolved by simple intake measures, because increased intake alone would support either interpretation of the drug effects.

Berridge was the first to apply a taste reactivity paradigm developed by Grill and Norgren to the problem of benzodiazepine induced hyperphagia, (Berridge & Treit, 1986). Treit and
Berridge tested the effects of intraperitoneal (ip) injections of the prototypical benzodiazepine chlordiazepoxide (CDP) in rats. Taste reactivity to sucrose, HCl and quinine was analyzed. A 10 mg/kg dose of CDP was chosen based on thresholds found in previous studies and the tastant solutions were kept low to avoid ceiling effects. Analysis revealed that CDP increased ingestive actions for all tastants and suppressed aversive reactions to HCl. Mild aversive reactions to sucrose, and strong aversive reactions to quinine were unaffected. This change in taste reactivity to a more positive response profile leads to the conclusion that GABA is involved directly in the biological modulation of palatability. Such a conclusion opens new direction for research on neurological mechanisms for feeding.

Building on that pioneering study, Berridge and Treit sought to strengthen their conclusion by determining whether the benzodiazepine antagonist Ro 15-1788 and inverse agonist CGS 8216 interfere with the enhancement of palatability by CDP (Treit & Berridge, 1986). Inverse agonists are so-called because they produce the opposite behavioral effects of agonists (Braestrup et al., 1982). Antagonists merely block the effect of agonists. CGS 8216 alone had been shown to have anorectic effects in rats, reducing intake in food-deprived rats (Cooper et al., 1985). The researchers compared taste reactivity following IP injections of saline, CDP, Ro 15-1788, CGS 8216, and combinations of CDP and each compound across two phases. They found that treatment with CDP in combination with either Ro 15-1788 or CGS 8216 produced no significant changes in taste affect over baseline. The results of this study twice replicated that of Berridge’s 1985 study in finding an effect of CDP on palatability. CGS had no intrinsic effect on positive or aversive reactions. Ro 15-1788 alone increased hedonic reactions to sucrose, suggesting that in the particular case of palatability, Ro 15-1788 is a mixed agonist/antagonist. When considered with the evidence of CDP’s effect, the data further support
the conclusion that a GABA/benzodiazepine-receptor-complex is directly involved in mediating palatability.

Following the success of their first two studies demonstrating the impact of benzodiazepines on palatability, Berridge and Treit next applied the taste reactivity paradigm to a comparison of the effect of benzodiazepines to those of dopamine and serotonin (1990). Dopaminergic agonists and lesions of dopamine systems had been found to suppress food and fluid intake in rats (Muscat et al., 1986). Serotonergic agents had been shown to facilitate ingestion (Dourish et al., 1985). The new study compared the effects of the benzodiazepine diazepam to those of the dopamine agonists apomorphine, d-amphetamine and antagonist haloperidol and to the 5-HT₁A agonists buspirone and gepirone (Treit & Berridge, 1990). Diazepam was the only experimental treatment that increased hedonic reactions to sucrose. Dopaminergic agents had no effect on hedonic or aversive reactions. Serotonergic agents suppressed both hedonic reactions and high levels of aversive reactions. Reactions to tastants eliciting low baseline levels of aversion were not affected by buspirone or gepirone. Consequently, the researchers concluded that the effect of benzodiazepines on increasing palatability is unique.

With the accumulation of evidence for the effect of benzodiazepines on hedonic palatability, the location of the site of action was the next crucial pursuit. Sites such as the cortex, hippocampus and corpus striatum were thought to be the most likely candidates, due to high concentrations of GABA receptors (Berridge, 1988). With a low density of GABA receptors, the brainstem was considered the least likely candidate. However, given that GABA had been implicated in number of mechanisms for feeding in the midbrain and hindbrain, Berridge
reasoned that a paucity of GABA receptors in the brainstem did not necessarily indicate a lack of importance (Scheel-Kruger et al., 1977; Berridge, 1988).

In a new study, the taste reactivity paradigm was applied to chronic mesencephalic decerebrate rats to test the effects of CDP in the hindbrain (Berridge, 1988). A reduced dose of CDP was administered to the decerebrate rats, because the sedative effects of CDP were found to be much stronger in that group. Despite this difference, the pattern of change in behavior from baseline resulting from CDP did not differ between decerebrate and non-decerebrate rats. This result indicates the midbrain/hindbrain alone has the capacity to increase palatability. To Berridge, these results supported the conclusion that the benzodiazepine receptor GABA receptors involved in the potentiation of food reward are at the midbrain or below, as are the neural circuits for ingestive behavior.

The study with decerebrate rats provided strong evidence in favor of a brainstem location of the site of action of benzodiazepines on palatability. To address the concern that the brainstem might not be the sole integrator in an intact brain, Peciña and Berridge (1996) next compared the effects of microinjections of diazepam in the fourth ventricle versus the lateral ventricle. They measured both feeding and taste reactivity following intraventricular (iv) microinjections of several different doses of diazepam. The results of the two experiments found that threshold doses between 40 and 50 μg in the fourth ventricle significantly increased feeding above baseline and increased hedonic taste reactivity significantly more than did injections in the lateral ventricle. The lateral ventricle did produce changes in taste affect at much higher doses, with a threshold around 75 μg. The researchers explained this disparity as an effect of diffusion of the drug from the lateral ventricle. They saw a clear overall effect of the fourth ventricle injections.
and concluded that the brainstem mediates the palatability enhancement by benzodiazepines, even in an intact brain.

In the late 1990s, Berridge briefly abandoned the brainstem for a comparison of benzodiazepines in the nucleus accumbens (NAc) shell. Accumulating evidence suggested that direct GABA agonists, glutamate antagonists, and opioid agonists act in the NAc shell to increase feeding. The NAc is an important site for pleasure, reward, and addiction. Particularly of interest, muscimol was shown to increase feeding in the NAc shell. Muscimol is a GABA_A agonist, but unlike benzodiazepines, it binds directly to the GABA site. Söderpalm and Berridge (2000a) sought to determine whether the shell of the NAc is similarly involved in benzodiazepine-potentiated hyperphagia. The study compared food intake following microinjections in the NAc shell. The effects of three compounds were examined: muscimol, morphine and diazepam. Several experiments were necessary to find appropriate dosages of muscimol and diazepam. Morphine increased food intake by 150%. Moderate doses of muscimol more than doubled food intake, but higher doses inhibited feeding by increasing agitation and exploration. A wide range of diazepam doses failed to produce any change in eating. As a final experiment, a dose of diazepam within the experimental range was injected into the fourth ventricle to verify an effect of benzodiazepines there; it significantly increased food intake. With these results, a role of the NAc in feeding behavior affected by muscimol and opioid agonists was confirmed; however, the lack of effect by diazepam led Berridge back to the brainstem.

The next step was to locate a site of action for benzodiazepines. Cooper and Higgs (1996) found that microinjections of midazolam in the parabrachial nucleus (PBN) of the pons increased food intake in rats. Following these results, Berridge and Söderpalm (2000b) measured food intake in a comparison of the effects of microinjections of diazepam to multiple brainstem sites.
implicated in the gustation or reward pathways. The PBN and the nucleus of the solitary tract are the secondary and primary nuclei of the gustatory pathway. The pedunculopontine tegmental nucleus plays a role in mediating food reward (Stefurak & Van der Kooy, 1994). Only injections to the PBN increased intake. A second experiment was performed, using taste reactivity measures to examine the effects of diazepam microinjections to the PBN. Positive ingestive reactions were increased to a bittersweet quinine/sucrose solution, the only tastant tested. From this can be concluded that the PBN mediates a majority, if not all, of the effect of benzodiazepines on palatability.

With just one possible central site of action located, much was as yet unknown regarding the chain action of benzodiazepines to increase food intake, in the PBN or elsewhere. As early as 1981, the effects of benzodiazepines on ingestive behavior had been suggested to depend on endogenous opioid activity (Britton et al., 1981). Cooper and Higgs (1994) first noted the similarity of effects of benzodiazepines and opioids on ingestive behavior; both increase intake, enhance preferences and increase positive ingestive reactions. Diazepam injection in rats had been found to induce changes in endorphin levels (Duka et al., 1980). And opioid antagonists, such as naltrexone, had been shown to block benzodiazepine-induced hyperphagia (Britton et al., 1981).

Berridge explored the possibility that benzodiazepines act to increase palatability by activating endogenous opioid peptides (Richardson et al., 2005). Ip injections were favored in this case, despite preceding work in localizing the individual effects of GABA and of opioids. Iv injections would require cannulae implantations at either the NAc or the PBN, or both; therefore ip treatment allowed for ease of administration. Furthermore, in the initial question of whether or not opioid antagonists will alter the effect of benzodiazepines, location of action was not the
focus. The effects on palatability by ip injections of diazepam, naltrexone and a combination of diazepam and naltrexone were compared using the taste reactivity paradigm. Diazepam predictably increased positive reactions by nearly double, while significantly suppressing aversive reactions. Naltrexone alone had no effect on either ingestive or aversive behaviors. In combination, diazepam and naltrexone had no effect on positive reactions, but exerted half the suppressive effect on aversive reactions as diazepam alone. It is unclear from these results what exact chain of neural events and structures is involved in this effect; however, Berridge concluded that the effect of benzodiazepines on palatability relies at least in part on endogenous opioid circuits.

_Opioids/the nucleus accumbens_

Opioid agonists had been shown on their own to induce hyperphagia, as with β-endorphin, in otherwise sated rats. In contrast, opioid antagonists reduce intake. Naloxone was found to suppress feeding even in food-deprived rats. Despite extensive research into the phenomenon, as of 1992 the mechanism by which opioids affect intake was not agreed upon. One hypothesis concerned an alteration of food palatability, but as with benzodiazepines in Berridge’s 1986 study, most of the previous evidence was based on food intake measures of preference.

Doyle, Berridge & Gosnell (1993) used a taste reactivity procedure to explore the effects of ip morphine on palatability. In a pilot experiment, food intake measures were used to determine the time at which morphine would achieve its peak effect for use in the taste reactivity procedure. In the experiment proper, the effect of morphine on palatability of the bittersweet sucrose/quinine solution was found to be unidimensional. Morphine significantly increased positive ingestive reactions, but aversive reactions were not affected. This lack of effect on
aversive reactions is in contrast to the palatability shift achieved by benzodiazepines. However, baseline aversive reactions may have been too low to establish significance. In summary, the results suggested that, as with benzodiazepines, opioids act to increase food intake by potentiation the hedonic impact of food.

Although evidence was converging to support the hypothesis that opiates act on food intake by shifting palatability, all the experiments had relied on systemic morphine administration. Hyperphagia had been shown to be induced by microinjections of morphine into the forebrain lateral ventricle. Peciña and Berridge (1995) conducted a new taste reactivity experiment administering iv microinjections of morphine to the lateral ventricle and testing reactions to sucrose. Both intake and hedonic reactions were potentiated by morphine, leading the researchers to conclude that the effect is centrally mediated.

To further pinpoint the site of action, Berridge and Söderpalm (2000) compared the effects on food intake of microinjections in the NAc shell. As discussed above, three compounds were examined: muscimol, morphine and diazepam. Morphine increased food intake by 150%. Moderate doses of muscimol increased food intake by more than double. No effect of diazepam was found. The main focus of the study was to determine what role, if any, the NAc plays in mediating benzodiazepine-induced hyperphagia. No evidence of such a role was found; however, these results did suggest a role of the NAc in feeding behavior affected by muscimol and by opioid agonists.

Similar local microinjection studies implicated many brain structures as potential mediators of hedonic palatability, including the nucleus accumbens, amygdala, striatum, hypothalamus, tegument, and hindbrain. However, all such reports were based on food intake measures (Peciña & Berridge, 2000). While systemic and iv morphine had been shown to
increase palatability through taste reactivity measures, simple food intake measures of the effect of morphine in specific sites do not rule out a varied effect of opiates. Morphine could act on one site to increase palatability while acting on another to increase food intake through a different mechanism. In a taste reactivity procedure for comparing the effects of microinjections of morphine, Peciña and Berridge focused on the NAc (2000).

Two problems arose from such a design. First, the core and the shell of the NAc differ widely in function and projection. Secondly, although the physical extent to which a drug diffuses from its injection site may be tracked, it is harder to know how much any activation may spread. To address these issues, the study employed a c-Fos mapping technique. C-Fos is a transcription factor, encoded by the Fos gene, which is rapidly expressed when neurons fire action potentials. By tracking upregulation of c-Fos mRNA, changes in neuronal activity can be mapped.

First, to assess whether the hyperphagia induced by morphine was actually mediated by opioid receptors, the researchers tested whether ip injections of naloxone would block the effect of intra-accumbens morphine. Then taste reactivity measures were used to confirm that microinjections of morphine potentiated the palatability of sucrose and quinine. Morphine increased food intake in the shell but not the core of the nucleus accumbens and was counteracted by systemic naloxone--indicating the involvement of opioid receptors. Finally, each subject was compared to itself measuring Fos expression between each hemisphere following unilateral morphine microinjection. This with-in subjects design was made possible by the finding in pilot studies that Fos expression following unilateral microinjections was similar to that in bilateral microinjections. They found that in either case, Fos expression exceeding 200% of the baseline was reliably indicative of morphine activity.
Histological treatment of brain tissue following the experiment indicated elevated Fos expression, creating a visual plume of activity. Each plume illuminates an area of activation by morphine. By combining all those plumes that also increased food intake by at least 133%, the researchers were able to map the site responsible for opioid-induced hyperphagia. They found a ‘hotspot’ in the medial caudal subregion of the NAc is primarily responsible for mediating the effects of intra-accumbens morphine. The site in question receives input from the amygdala and has axonal projections to the ventral pallidum, substantia innominata and to the pontine parabrachial nucleus (PBN). The ventral pallidum and substantia innominata are implicated in food reward, and the PBN is an important gustatory relay in rats. The relationship between these sites implies that opioids are involved in food reward by mediating palatability through the PBN. Overall, the findings of the 2000 study suggest that the medial caudal subregion of the NAc plays a functional role in mediating food reward by increasing the hedonic impact of food.

The c-Fos procedure as a method of mapping has a few limitations. Fos is not universally expressed by all neurons. Furthermore, it is not clear whether the Fos expression tracked was from neurons directly stimulated by morphine or from secondary neuron activation by neurons with morphine receptors. Finally, within a neuron that does express Fos, expression of the gene is extremely variable over time. In that regard, the activity Berridge and Peciña were able to map was limited by the time at which they chose to sacrifice the subjects.

In 2005, Peciña and Berridge repeated the c-Fos mapping procedure of their 2000 study, but used the μ-opioid agonist d-Ala²-N-Me-Phe⁴-Gly⁵-ol-enkephalin (DAMGO) instead of morphine. In the previous study, a map was achieved by combining the Fos plumes resultant from microinjections that induced hyperphagia. In the new study microinjections were focused on the medial NAc shell, and separate maps were created for increased palatability versus
increased intake alone. Microinjections of DAMGO resulted in increased food intake. However, potentiation of hedonic impact of sucrose by DAMGO was restricted to a small site in the rostroventral subregion of the medial NAc shell. These results are important not only in the identification of a site responsible for increasing palatability, but also in demonstrating a difference between mediation of food intake and hedonic impact.

In addition to this evidence of opioid mediation of palatability, opioids had been shown to interact with benzodiazepines in the latter’s effect on taste. Furthermore, opioid receptor antagonists such as had been found to block selectively the hyperphagic effects of benzodiazepines.

Berridge explored the possibility that benzodiazepines act to increase palatability by activating endogenous opioid peptides (Richardson et al., 2005). The effects on palatability by ip injections of diazepam, naltrexone and a combination of diazepam and naltrexone were compared using the taste reactivity paradigm. Diazepam predictably increased positive reactions by nearly double, while significantly suppressing aversive reactions. Naltrexone alone had no effect on either ingestive or aversive behaviors, however, in combination, diazepam and naltrexone had no effect on positive reactions while exerting half the suppressive effect on aversive reactions as diazepam alone. The exact chain of neural events and structures involved in this effect is not clear from the 2005 study. With his overall research on opioids and ingestive behavior, Berridge confirmed the importance of opioid circuits in mediating the effect of benzodiazepines on the hedonic impact of food. Furthermore, he helped identify a possible site of action for opioids in the NAc shell.

\textit{Dopamine}
Lesions and pharmacological blocking of mesostriatal dopamine-containing neurons had been shown to suppress feeding and drinking (Muscat et al., 1986). Attempts to explain these deficits were previously of two kinds (Berridge et al., 1989). One kind explained the effect as decreased sensorimotor arousal resulting in a lowered ability to respond to stimuli. The second type emphasized anhedonia as a result of dopamine depletion, indicating a decrease in the pleasurable impact of stimuli.

Berridge, Venier and Robinson (1989) designed an experiment to test the two competing hypotheses using taste reactivity measures in rats. If the aphagia and adipsia in lesioned rats were due to an overall attenuation of sensorimotor arousal, then both ingestive and aversive reactions would be suppressed. If, however, anhedonia were responsible, then ingestive reactions would be selectively suppressed. Intranigral injections of 6-hydroxydopamine were administered to lesion selectively the mesostriatal dopamine system, following pretreatment with desipramine, a norepinephrine (NE) reuptake inhibitor, to protect cells containing norepinephrine. Due to noted gastrointestinal effects of the NE reuptake inhibitor, both the saline control and the 6-OHDA experimental groups included a group that received saline pretreatment instead. No difference was found between desipramine and desipramine-control groups.

Following surgery, rats that received 6-OHDA were divided for analysis into two groups based on spontaneous food intake, hypophagic and aphagic. Among all subjects, no effect of treatment or intake group was found on taste reactivity. Taste was the only factor that affected ingestive or aversive behavior, with no interactions. In a secondary analysis of response likelihood, no effect of tastant concentration was found, indicating that stimuli thresholds had not been raised. These results support neither hypothesis; however, an important implication of the study is the dissociation of palatability from motivation.
Although Berridge’s work supported neither hypothesis, evidence in support of an anhedonia hypothesis continued to mount (Smith, 1995). In particular, taste reactivity studies by Linda Parker particularly found contradictory results to Berridge’s work. Parker found that pimozide, a dopamine antagonist that also moderately inhibits dopamine reuptake, suppressed hedonic reactions to sucrose and enhanced aversive reactions to quinine in the second halves of longer taste infusions. To resolve the controversy, the two labs combined in a new study that addressed several differences in methodology between the two labs (Peciña et al., 1997).

One lab performed a within subjects design and the other made comparisons between subjects, but the methodologies otherwise matched. Taste reactivity to a long-term sucrose infusion was scored following ip injections of pimozide. The experiments were repeated with a solution of quinine as the tastant. An important modification in the analysis by this study as compared to previous studies by Parker was to adjust the number of behaviors recorded relative to the time the subjects were actually visible on camera. The immediate initial reactions were unchanged, consistent with Berridge’s previous findings. Pimozide was found to suppress both positive and aversive reactions after several minutes of repeated exposure to the tastant in question. The findings ultimately support a sensorimotor-based explanation for the aphagic effect of dopamine disruption.

Dopamine agonists had also been shown to suppress feeding. Given the mounting evidence from his own lab that benzodiazepine-induced hyperphagia is a result of direct enhancement of palatability, Berridge next explored the possibility that dopamine agonists acted to suppress food intake by a similar but opposing mechanism. As discussed above, Treit and Berridge (1990) compared the effects on taste reactivity of the benzodiazepine diazepam to those of the dopamine agonists apomorphine, d-amphetamine and antagonist haloperidol and to the 5-
HT₁A agonists buspirone and gepirone. No effect of any dopamine agonist was found on either hedonic or aversive reactions.

Subsequently, Berridge used a genetic mutant approach to examining the role of dopamine in liking and reward (Peciña et al., 2003). Hyperdopaminergic mice were observed in a runway task and in a taste reactivity test in response to sucrose. The mice were found to learn faster and exhibit greater incentive performance—being less distracted from and proceeding more directly to the reward. However, ingestive reactions to sucrose were unchanged between normal and hyperdopaminergic mice. The implication of these results is that extra dopamine increases wanting or motivation without affecting the sensory liking for the reward.

**Steven J. Cooper & Suzanne Higgs**

Suzanne Higgs first came to collaborate with Steven J. Cooper when completing her Ph.D. in pharmacology under his mentorship. After she received her doctorate, they continued to cooperate on research. Their main area of interest was in the psychopharmacology of appetite. As with Berridge, the Cooper and Higgs examined benzodiazepines and other drugs. They used these pharmacological tools to investigate endogenous compounds and neural mechanisms involved in ingestive behavior.

To analyze palatability effects, they frequently employed a microstructural (MS) analysis of licking patterns. This method uses an automated licker system to measure multiple characteristics of lick data. Individual licks are counted, as well as latency to lick. The interlick interval is defined as the time between individual licks, and a bout of licking is defined as a group of licks during which the interlick interval does not exceed more than 400 ms. In addition
to producing a direct measure of ingestive behavior, the data gleaned from this approach also provides an indication of palatability. For example, whereas food deprivation increases the number of bouts, increased concentration of palatable substances increases mean bout duration (Spector et al., 1998). This effect indicates that length of bout duration is indicative of palatability.

Higgs and Cooper (1996a) first examined the effect of the partial inverse agonist Ro 15-4513 on the intake of sucrose and a sodium saccharine solution. To analyze the role of the benzodiazepine inverse agonist in hypophagia, intake was compared in terms of licking behavior. Unlike later applications of MS analyses, this earliest study depended on video recordings for collecting data rather than from an automated apparatus. The lick rate between bouts and the latency to begin drinking were unchanged by Ro 15-4513, indicating that neither overall appetite nor motivation was affected. Rather, decreased intake in the experimental groups was due to a decrease in initial lick rate; the researchers found that the total duration of drinking, total licks and number of bouts for both sucrose and saccharin were all significantly lower. These results supported the hypothesis that benzodiazepines increase food intake by increasing the palatability of food. By examining licking patterns over brief access testing of 30-60s exposure to each tastant, the method further prevents interference by gut feedback.

A second microstructural analysis of the effects of midazolam and Ro 15-4513 on licking behavior was performed using sucrose, Intralipid and maltodextrin (Higgs & Cooper, 1998a). Although similar, two key differences in methodology were introduced. First, the time of exposure to each tastant per trial was much shorter at 20s. Second, whereas the initial study used video recordings to record licking behavior, the new study employed specialized testing apparatus to digitally record licking behavior data. The effects of midazolam and Ro 15-4513
were opposite each other. Midazolam increased total licks by increasing bout duration. Ro 15-4513 decreased total licks by decreasing bout duration. As with the previous study, drug action at benzodiazepine receptors is interpreted to be an effect on palatability.

Building on Berridge’s work locating the role of GABA in the brainstem, Higgs and Cooper (1996b) confirmed the role of the brainstem in mediating benzodiazepine-induced hyperphagia using the benzodiazepine receptor agonist midazolam and the antagonist flumazenil. First, midazolam microinjections in the fourth ventricle resulted in increased ingestion of a palatable mash. In the second half of the study, ip administration of flumazenil was found to block the hyperphagic effects of intraventricular midazolam, indicating a role of specific benzodiazepine receptors in mediating the hyperphagic effects of benzodiazepines in the brainstem.

Cooper’s lab had completed multiple studies regarding the effect of benzodiazepines on sweet and fat ingestive behavior but not on salt. Cooper and Higgs (2005) thus pursued a microstructural analysis of licking response to NaCl solution in rats treated with ip midazolam. As with other tastants, it was found that midazolam increased the total number of licks by increasing mean bout duration, not by increasing number of bouts. From these results, the researchers concluded that the role of GABA in palatability enhancement of sweet and fat tastants generalizes to salt as well.

To investigate the PBN as a possible site of action in mediating benzodiazepines effect on ingestive behavior, Higgs and Cooper (1996c) measured food intake following microinjections of midazolam. Midazolam injected within 1mm of the PBN significantly increased food intake, by almost double. Location within the PBN had no effect. Intake of a sucrose solution was also increased. Additionally, systemic flumazenil administered slightly
before treatment with midazolam greatly attenuated the effect on intake. The implication is that benzodiazepine receptors in the PBN are the site of action for benzodiazepine-induced hyperphagia. Furthermore, observation of motor activity found no effect of PBN midazolam, indicating that the effect of benzodiazepines in the PBN is specific to ingestive behavior.

With a likely central site of action identified, it became crucial to identify the chain of action within which benzodiazepines and the PBN ultimately mediate hyperphagia. As previously discussed, endogenous opioids had been implicated as necessary to benzodiazepine effects. In a new study, a microstructural analysis of licking behavior was used to assess the possible role of endogenous opioids in the hyperphagic effects of midazolam (Higgs & Cooper, 1997). As with their previous studies, access to each tastant was limited to 60s. The effects of flumazenil and naloxone were compared in attenuating the midazolam-induced licking pattern responses to Intralipid. Midazolam acted to increase the total number of licks by increasing the mean bout duration. This effect occurred despite a decrease in intrabout lick rate, which was indicative of the sedative effect of the drug. When administered alone, neither flumazenil nor naloxone produced any effect; however, both drugs blocked the increase of mean bout duration induced by midazolam. Where the drugs differed was in decreasing intrabout lick rate. Altogether, the data suggest that endogenous opioids are involved in the effects of midazolam on palatability but not in those on sedation.

As with opioids, Higgs and Cooper (2000) next used dopamine antagonists as a pharmacological tool for examining the neurochemical mechanisms involved in the effect of GABA on ingestive behavior. A role of dopamine had been implicated in the benzodiazepine-induced enhancement of palatability. For example, Schneider et al. (1986) demonstrated that the D₂ dopamine receptor antagonist raclopride induces an effect on pattern of intake similar to
midazolam but in the opposite direction. They found that raclopride-treated rats exhibited a lower overall intake of sucrose as a result of shorter licking bouts. Higgs and Cooper pretreated rats with 0.1 mg/kg raclopride. Pilot studies found this dose to be too low to achieve behavioral effects. Rats were then treated with ip midazolam, and microstructural analysis of licking response to sucrose was performed. Raclopride blocked the benzodiazepine effect of increasing bout size but failed to modulate the benzodiazepine effect of decreasing intrabout lick rate. Thus, dopamine differentiates the benzodiazepine effects of increasing bout duration and decreasing lick rate. Such a differentiation supports the hypothesis that decreased intrabout lick rates in benzodiazepine-treated rats are due to motor effects of the anxiolytics. More importantly, the data suggests that the effects of GABA on palatability are at least partially dopamine-dependent.

Higgs and Cooper also briefly examined compounds other than benzodiazepines for involvement in modulating ingestive behavior. Similar to Berridge, they sought to determine a role of endogenous opioids (Higgs & Cooper, 1998b). In research on opioid-induced hyperphagia, much effort had been applied in identifying the receptor subtypes involved in modulation of ingestive behavior. Little focus had been devoted to explicating the behavioral changes induced by activity at each receptor subtype. Thus, Higgs and Cooper applied a microstructural analysis of licking response to sucrose and Intralipid following ip treatment with different opioid ligands. Naloxone was used as a nonselective opioid antagonist. Morphine was chosen as a μ-opioid agonist, and U-50,488H was used to examine the effects of a selective κ-opioid receptor agonist.

In general, morphine and naloxone were found to have opposite effects. Naloxone decreased total number of licks and number of bouts in response to both tastants. Morphine increased total number of licks and number of bouts. The only divergence from this pattern was
the effect of morphine to decrease mean bout duration in response to Intralipid, whereas naloxone had no effect on mean bout durations. Similar to morphine, U-50,488H increased both the total number of licks and the number of bouts. However, the κ-opioid receptor agonist also increased the mean bout durations. Furthermore, whereas the effects of naloxone and morphine increased with increasing doses, higher doses of U-50,488H did not significantly affect any dependent variable. These data do not indicate a unifying neurochemical mechanism underlying the behavioral effects of opioids receptor subtypes. In other words, the behavioral effects differentiate the roles of each subtype in modulating ingestive behavior.

Another class of chemicals examined was neuroactive steroids. A neuroactive steroid is a steroid that can be synthesized in the brain from cholesterol (Baulieu, 1981). They had been shown to act at GABA_A receptors (Gee, 1988). The neuroactive pregnane steroid 3α-hydroxy-5β-pregnan-20-one (pregnanolone) is a positive modulator of GABA transmission. Its behavioral effects are similar to benzodiazepines, including hyperphagia of sweet tastants by rats (Chen et al., 1996). Higgs and Cooper (1998c) analyzed ip pregnanolone-treated rats’ licking responses to sucrose solutions, as well as measuring intake of both novel and familiar mash preparations. The researchers found that pregnanolone had no effect on lick measures or on intake of the familiar mash. However, treatment with pregnanolone resulted in significantly more intake of the novel mash. The researchers conclude that pregnanolone may modulate increased ingestion indirectly through an anxiolytic effect.

**Discussion**

Kent Berridge and the collaboration of Steven J. Cooper and Suzanne Higgs have contributed much to the current understanding of rat taste. Like the different methods used to assess
palatability, the two labs are complementary in their illumination of ingestive behavior. When the results of Berridge’s taste reactivity paradigm studies are considered along with the microstructural lick analyses of Higgs and Cooper, a strong conclusion arises that GABA is directly involved in palatability. Furthermore, their efforts have begun to identify a site of action and several possible mechanisms through which the effect of GABA may be achieved. These research discussed heavily indicates that GABA most likely acts in the parabrachial nucleus of the pons to increase food intake by potentiating the hedonic impact of food.

In the case of both Berridge’s work and the work of Cooper and Higgs, initial research into opioid effects on palatability have led to an increased understanding of the role of GABA in modulating ingestive behavior through palatability. Supported by both labs, the opioid-dependence of GABA influence on hedonic impact is particularly telling in light the site of opioid action identified by Berridge. The interconnection of the nucleus of accumbens with the PBN and sites heavily implicated in reward is an exciting first clue to identifying the adaptive value of a role of GABA in taste and ingestive behavior. Whereas neuroscientists manipulate GABA with benzodiazepines, GABA in the PBN may be manipulated naturally by the reward system. A more traditional notion of reward is that changing the internal value of a stimulus modifies the behavior associated with that stimulus. In contrast, the cumulative work of Berridge, Cooper and Higgs hints at a reward system that also modulates behavior by directly enhancing the hedonic impact of the stimulus itself.

One area on which Cooper and Higgs differed from Berridge was in their conclusions regarding the role of dopamine in increasing food intake. Berridge’s taste reactivity studies concluded that dopamine increased the value of palatable tastants without affecting their hedonic impact. In other words, dopamine agonists increased wanting of a stimulus without increasing
liking of it. From microstructural (MS) analyses of licking patterns, Higgs and Cooper concluded that dopamine is crucial to the effect of GABA on palatability. This contrast indicates a possible shortcoming of the assumptions of MS analyses. Cooper and Higgs interpret mean bout duration as a direct measure of palatability. However, it is possible that mean bout duration instead measures motivational response to palatability. This interpretation would fit with Berridge’s incentive-salience hypothesis of dopamine function but would carry heavy implications for the conclusions from all other MS analyses. More research is needed to resolve the role of dopamine in ingestive behaviors.

As with dopamine and reward pathways, the contributions of Kent Berridge and of Steven Cooper and Suzanne Higgs to the field of taste research have opened new, as yet unexplored areas of research. Any new avenues of exploration opened by the research reviewed here are not questions left unanswered so much as they are leads waiting to be followed. In this way, the overall effect of the works of both Berridge and also Higgs and Cooper is an incredible contribution to the wider body of knowledge regarding taste and ingestive behavior.
Works Cited


