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Authors
Wheeler, Robert A.
Roitman, Mitchell F.
Grigson, Patricia S.
et al.

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Single Neurons in the Nucleus Accumbens Track Relative Reward

Robert A. Wheeler, Mitchell F. Roitman,
University of North Carolina, Chapel Hill, U.S.A.

Patricia S. Grigson, and
Penn State University College of Medicine, U.S.A.

Regina M. Carelli
University of North Carolina, Chapel Hill, U.S.A.

A within subjects simultaneous contrast experiment evaluated nucleus accumbens (NAc) neural responses to a low (0.05 M) and high (0.5 M) concentration of sucrose in 6 rats. During continuous trials, rats were given repeated brief intraoral infusions of the low and then the high concentration of sucrose while electrophysiological activity of NAc neurons and oromotor behavior (EMG) were measured. Following the continuous phase of testing, the two concentrations were infused in an alternating manner. The results showed that rats reduced oromotor behavior when infused with the low concentration of sucrose when alternated with the high concentration (i.e., during alternating trials) relative to the infusion during the continuous low condition (negative contrast). Rats also increased oromotor behavior for the high concentration when presented during alternating relative to continuous trials (positive contrast). Of 137 NAc neurons, 35 exhibited brief inhibitions or excitations to tastant delivery during baseline testing that were correlated with oromotor output. Some NAc neuronal activity reflected negative or positive contrast effects while other neurons encoded alternating testing in general and still other neurons encoded sucrose concentration. These data demonstrate that neuronal activity in the NAc is altered in coincidence with the expression of contrast in consummatory behavior.

Rewarding stimuli are not procured and consumed in a vacuum. Rather, traces of previously rewarding experiences alter the perceived value of a current reward. Flaherty recognized this and greatly contributed to the characterization of contrast phenomena in consummatory behavior (Flaherty & Checke, 1982; Flaherty & Grigson, 1988; Flaherty & Rowan, 1986; Flaherty, Turovsky, & Krauss, 1994). In the most basic form of consummatory contrast, rats will reduce intake of an otherwise palatable sucrose solution when alternated with a more preferred sucrose solution than when presented alone. Likewise, rats will increase intake of a more palatable sucrose solution when alternated with access to a less preferred sucrose solution than when the high concentration is presented alone. These phenomena, known respectively as simultaneous negative and positive contrast effects, are thought to reflect a rapidly modified relative reward coding (Flaherty, 1999).

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Previous investigations of the neural basis of simultaneous contrast have focused on the contribution of the brainstem (Grigson et al., 1997) while the role of the most extensively studied system in reward processing, the mesolimbic system, remains unclear. Individual neurons within the nucleus accumbens (NAc) modulate their firing rates with respect to the reward associated with food (Nicola, Yun, Wakabayashi, & Fields, 2004b; Roitman, Wheeler, & Carelli, 2005; Roop, Hollander, & Carelli, 2002) and drugs of abuse (Carelli & Wightman, 2004). Moreover, NAc responses are highly plastic and are altered by manipulations of learned associations (i.e., extinction training; Carelli & Ijames, 2000; Hollander, Ijames, Roop, & Carelli, 2002). Here, we sought to determine if individual NAc neurons respond differentially to the same concentration of sucrose when presented alone or in alternation with a different concentration of sucrose—that is, whether NAc neurons exhibit a neural correlate of simultaneous negative and positive contrast. Rats were exposed to continuous and alternating intraoral infusions of a low (0.05 M) and a high (0.5 M) concentration of sucrose in a within subjects simultaneous contrast design. During this paradigm, the electrophysiological activity of individual NAc neurons was simultaneously recorded with the electromyographic (EMG) activity of the anterior digastric—a muscle whose activity is coupled to licking (Roitman et al., 2005; Kaplan, Roitman, & Grill, 1995; Travers & Norgren, 1986). The results indicate that NAc responses to sucrose are indeed plastic and depend on the context in which they are presented.

Method

Subjects

Six male Sprague-Dawley rats (weighing between 280-350 g) were individually housed with ad libitum food and water with a 12:12 light:dark cycle (lights on at 07:00 h). All experiments were conducted in the light phase of the cycle between 10:00 and 15:00 h.

Surgery and Histology

Rats were anesthetized with a ketamine (100 mg/kg)-xylazine (20 mg/kg) mixture. For EMG recordings, the uninsulated tips of 2, 7-strand stainless steel wires (A-M Systems, Carlsborg, WA) were implanted into the anterior digastric muscle and the other ends were led subcutaneously out an incision in the top of the head where they mated with an omnetics connector, adapted from Kaplan et al., (Kaplan et al., 1995). Another wire wrapped around a skull screw served as ground for EMG recordings. For electrophysiological recordings, 8-wire microelectrode arrays (N-B Labs, Dennison, TX) were implanted bilaterally in the NAc. The coordinates used, in accordance with the atlas of Paxinos and Watson (Paxinos, 2005), were AP: +1.7 mm, ML: ±0.8 to ±1.3 mm, DV: -6.5 mm from surface. For each array, another wire was wrapped around a skull screw to serve as ground. Finally, rats were bilaterally implanted with intraoral cannulae. Each cannula consisted of an approximately 6cm length of PE-100 tubing which was phalanged at one end with a Teflon washer. The cannula was inserted just lateral to the first maxillary molar with the Teflon washer flush against the molar. The other end was exteriorized out the incision at the top of the head and held in place along with the EMG connector and arrays with dental acrylic. Rats were permitted at least 1 week to recover from surgery.

Following experiments, rats were deeply anesthetized with ketamine/xylazine and electrode tips were marked by passing current (10 µA, 5 s) through the electrodes. Rats were then transcardially perfused with phosphate buffer and 4% paraformaldehyde, brains were removed and, after post-fixing and freezing, sliced into 40 µm sections through the forebrain. Sections were then

mounted on slides and stained with potassium ferocyanide and counterstained with thionin to visualize electrode tips.

Electromyographic and Electrophysiological Recordings

Electrophysiological procedures have been described in detail previously (Carelli & Deadwyler, 1994; Carelli, Ijames, & Crumling, 2000). Briefly, before the start of the behavioral session, the rat was placed into a plexiglas chamber within a sound attenuating box. Rats were connected to a flexible recording cable (Plexon, Texas, U.S.A.) attached to a commutator (Med Associates, Vermont, U.S.A.) that permitted virtually unrestrained movement within the chamber. The headstage contained 16 miniature unity-gain field effect transistors. NAc activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (multichannel acquisition processor (MAP) system; Plexon, Texas, U.S.A). Multiple window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals on the basis of waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal spike events to a Pentium computer. Another computer controlled behavioral events of the experiment (Med Associates, Vermont, U.S.A.) and sent digital outputs corresponding to each event to the MAP box to be time-stamped along with the neural data. The neurophysiological system has the capability of recording up to four neurons per microwire using real-time discrimination of neuronal action potentials. However, in the present study, typically one or two neurons were recorded per microwire, as in previous reports (Carelli & Deadwyler, 1994; Carelli et al., 2000). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (Carelli et al., 2000). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template analysis procedures provided by the neurophysiological software system (MAP system). The template analysis procedure involves taking a "sample" of the waveform and building a template of that extracellular waveform. Subsequent neurons that "match" this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment was over using principle components analysis in Offline Sorter (Plexon, Texas, U.S.A.). After sorting, firing rates of individual neurons were aligned to pump onset for the intraoral infusion. Perievent histograms were constructed using commercially available software (NeuroExplorer, Plexon, Texas, U.S.A.). Firing rates were calculated for each neuron across 1-s intervals from -8 to +8 s relative to pump onset in 100 ms bins. Data were then imported into Excel.

For EMG recordings, rats were attached to a second flexible cable (Plexon, Texas, U.S.A.) and EMG potentials were recorded differentially. Briefly, wires were led to an amplifier (Plexon, Texas, U.S.A.) and signals were amplified (1000X). Processed analog signals were then led through a national instruments board to the same computer that collected electrophysiological data. The same program (Sort Client, Plexon, Texas, U.S.A.) that collected electrophysiological data also collected EMG data. To analyze EMG signals, a horizontal threshold was positioned higher than at least 3σ of the noise. Threshold crossings were time stamped and examined relative to pump onset in NeuroExplorer. Statistical analyses of both electrophysiological and EMG signals were performed using commercially available software (Statsoft, Oklahoma, U.S.A.).

Experimental Design

On the test day, naïve rats were placed in the recording chamber and were connected to EMG and electrophysiological cables as well as infusion lines to the 2 intraoral cannulae. After neurons were sorted on line, the experimental session began. Initially, the chamber was illuminated by a light on top of the rear wall of the chamber. After a 56 s delay, the continuous phase began when an infusion pump (model PHM-100; Med Associates, Vermont, U.S.A.) turned on for 4 s to deliver 133 µl of 0.05 M sucrose. Following the infusion, there was another 56 s delay before the next trial. After a block of 20 trials with 0.05 M sucrose, a second block of 20 trials was completed with 0.5 M sucrose. Fifty-six seconds following these trials, the alternation phase began with 0.05 M and 0.5 M sucrose being delivered in an alternating manner for a total of 20 trials each. The

order of stimulus delivery during the continuous trials was counterbalanced, with 3 rats receiving 0.5 M sucrose first and 3 rats receiving 0.05 M sucrose first.

Data Analysis

EMG. EMG activity of the anterior digastric muscle was measured while a low (0.05 M) or high (0.5 M) sucrose solution was infused into the oral cavity of the rat. Rats emit stereotypical oromotor responses to appetitive tastants (Grill & Norgren, 1978; Kaplan et al., 1995). We measured counts of threshold crossings of filtered EMG analog signals taken from activity of the anterior digastric muscle, an obligate jaw opener during ingestive behavior, as a measure of oromotor behavior elicited by low and high concentrations of sucrose. Behavioral contrast is typically defined by a reduction or increase in intake of a particular solution due to its sequential presentation with a different solution. Here we measured EMG activity of the anterior digastric muscle as a measure of oromotor behavior and hence intake. Mean EMG activity for each condition was analyzed. The mean activity during each 1-s bin from 0 (pump onset) to +8 s (4 s infusion, plus 4 s post infusion) was examined.

Determination of Phasic Responses. Neural activity was characterized via perievent histograms (PEHs) showing the activity of each neuron during a 40 s time period that bracketed sucrose delivery. Consistent with other reports from our lab (Carelli et al., 2000; Roitman et al., 2005; Roop et al., 2002), NAc neurons were classified as exhibiting a phasic excitation or inhibition in firing rate during continuous low and continuous high sucrose concentration delivery. Specifically, NAc neurons were classified as phasic (excitatory or inhibitory) to tastant delivery if their activity during the 8 s test period (4 s infusion, plus 4 s post infusion) deviated from baseline (mean firing rate from -8 to 0 s prior to pump onset) by at least 40%.

Neural Representation of Contrast Effects. After determination of phasic responses to continuous tastant delivery (i.e., absolute reward properties), we examined whether neurons showed differences in firing rate when the same tastant was delivered in an alternating fashion (i.e., during negative or positive contrast). In order to make this comparison, signal-to-baseline (S:B) ratios of each neuron's response to each of the 4 conditions was calculated. In this way, one value indicative of the magnitude of the neuron's responses for each condition could easily be compared for representations of contrast effects. Importantly, differences in baseline firing rates across NAc neurons (range: 0.70-16.37 spikes/s) are controlled for using S:B ratios. S:B ratios were determined by dividing the mean firing rate during the 8 s test period by the mean firing rate of the same neuron during the 8 s baseline period (i.e., the 8 s prior to stimulus infusion). An S:B ratio of 1 indicates no change in neural firing rate during the infusion period. S:B ratios less than 1 indicate neural inhibitions while S:B ratios greater than 1 indicate neural excitations. Contrast effects were then determined by statistical comparisons of these ratios (i.e., low continuous vs. low alternating), separately for neurons with excitatory responses and neurons with inhibitory responses. It was hypothesized that neural representations of contrast effects would resemble behavioral contrast effects. That is, an augmentation of the magnitude of the neural response would represent a positive contrast effect, and a reduction in the magnitude of the neural response would represent a negative contrast effect.

Results

Histology

Histological reconstruction of electrode placement showed that the neurons presented in this report were in the rostral pole, core or shell subregions of the NAc as defined by Zahm and Brog (Zahm & Brog, 1992). Electrode placements spanned a rostral caudal distance of 1.1 mm, ranging from 2.7 mm to 1.6 mm rostral to bregma and from 0.50 mm to 2.20 mm lateral to midline. A total of 96 wires were implanted in 6 rats. Of those 96, 6 wires were not located in the nucleus accumbens core, shell or rostral pole subregions and neurons recorded from these arrays were

excluded from the analysis. There appeared to be no difference in response types across core and shell subregions although subsequent experiments will explore this further. Only data from electrode placements within the borders of the NAc as depicted in the atlas of Paxinos and Watson (2005) are presented here.

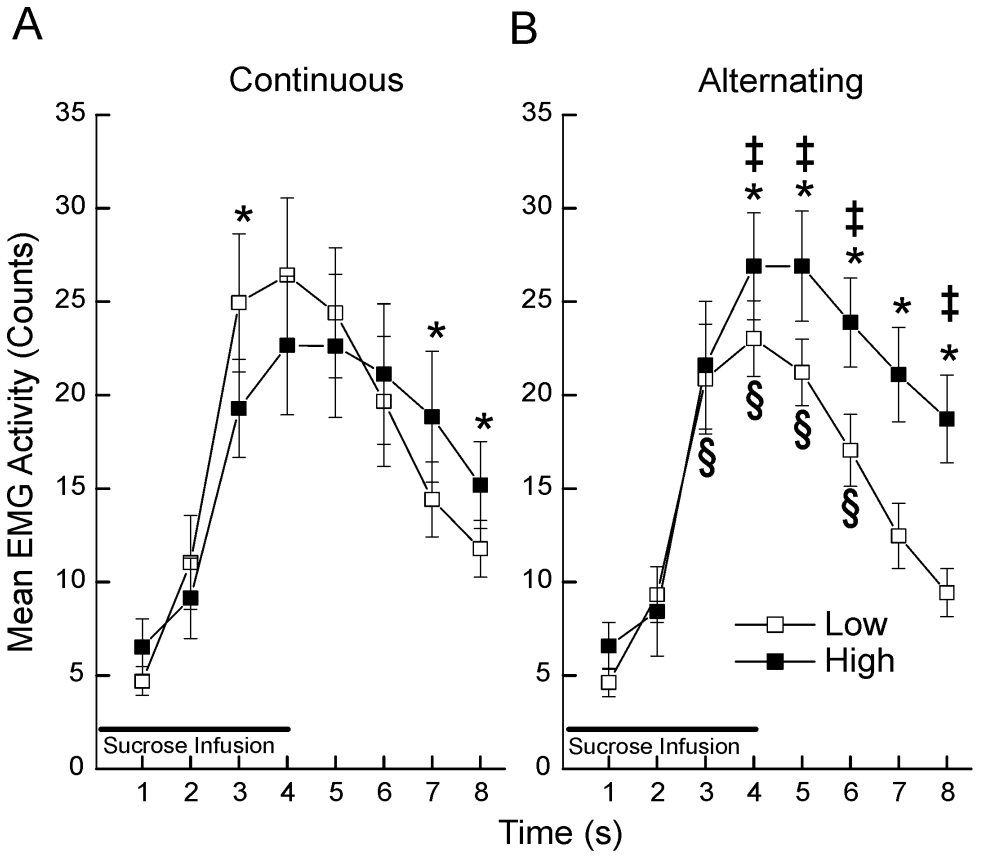

Figure 1. Simultaneous contrast. A. Mean (±SEM) EMG activity as measured by threshold crossing counts in 1 s bins during the continuous phase of the experiment. EMG activity was significantly greater for the high concentration of sucrose compared to the low only at time points 7 and 8 and greater for the low concentration than the high at time point 3. B. Mean (±SEM) EMG activity during the alternating phase. Significantly more EMG activity was elicited by the high concentration of sucrose compared to the low at time points 4-8. Asterisks indicate significant differences between low and high. ‡ indicates significant increase in EMG activity compared to high continuous (positive contrast). § indicates significant decrease in EMG activity compared to low continuous (negative contrast), $p < 0.05$.

EMG

Behavioral evidence of simultaneous negative and positive contrast was investigated using EMG recordings of the anterior digastric muscle (Figure 1). A 2 x 2 x 8 repeated measures ANOVA varying concentration (low and high), phase (continuous and alternating), and time (1-8 s bins) was performed on EMG activity. Newman Keuls post hoc tests were run on significant interactions. Intraoral sucrose infusions elicited concentration-dependent EMG activity. A significant main effect

of Concentration, $F(1, 5) = 10.17$, $p < 0.05$, indicated that infusions of the high concentration elicited more EMG activity relative to low, and this difference was apparent during the alternating phase of conditioning, see Figure 1B. Furthermore, Newman Keuls post hoc tests of a significant concentration x time interaction, $F(7, 35) = 7.77$, $p < 0.05$, confirmed that EMG activity was greater for high than low specifically after the infusion terminated (postinfusion period), see Figure 1. Most importantly, post hoc tests of a significant concentration x phase x time interaction $F(7, 35) = 2.39$, $p < 0.05$, revealed that rats exhibited both simultaneous negative, and positive contrast. Rats exhibited reduced EMG activity for the low concentration of sucrose during the alternating phase relative to the continuous phase at 3, 4, 5 and 6 s time points (i.e., negative contrast). Also, rats exhibited increased EMG activity for the high concentration of sucrose during the alternating phase relative to the continuous phase at the 4, 5, 6 and 8 s time points (i.e., positive contrast). These contrast effects are evident in Figure 1. During the continuous phase, EMG activity was significantly greater for the high concentration of sucrose compared to the low only at time points 7 and 8, see Figure 1A. At time point 3, more EMG activity was generated for the low concentration than the high. However, during the alternating phase, significantly more EMG activity was elicited by the high concentration of sucrose than the low at time points 4-8, see Figure 1B. Alternating delivery, then, resulted in an augmented magnitude of reward effect via both negative and positive contrast effects in consummatory behavior.

Electrophysiology

NAc neurons exhibit inhibitory or excitatory responses to intraoral infusions of sucrose. A total of 137 NAc neurons (mean baseline firing rate 4.16 spikes/s) were recorded in 6 rats. Of the 137 neurons, 35 exhibited phasic excitations or inhibitions during continuous tastant delivery. These neurons were further classified based upon their responsiveness to low and high sucrose delivery. For the low concentration of sucrose, 13 neurons exhibited inhibitory responses and 13 showed excitatory responses. For the high concentration of sucrose, 13 neurons showed inhibitory responses and 11 neurons showed excitatory responses. These classifications were not mutually exclusive. For example, a neuron with an excitatory response to the low concentration of sucrose often also displayed an excitatory response to the high sucrose concentration. The primary purpose of the present study was to determine whether NAc neurons changed their responsiveness to a particular concentration of sucrose when it was presented in an alternating fashion with a different concentration. As described below, populations of NAc neurons were indeed altered in this manner thereby reflecting both negative and positive contrast. Additionally, some neurons were sensitive to the pattern of stimulus testing in general (i.e., coded for the continuous vs. the alternating delivery of reward) but did not show negative or positive "directional" contrast effects. Finally, another population of neurons was sensitive to sucrose concentration, independent of contrast testing. Together these neurons encode for concentration effects during the alternating trials (augmented by negative and positive contrast), concentration effects during continuous (noncomparison) stimulus delivery, and for the pattern of continuous vs. alter-

nating stimulus delivery, per se. The types of neuronal responses are described in detail below.

Negative and Positive Contrast Effects

For the population of neurons that displayed inhibitory responses to the low concentration of sucrose in the continuous phase of the experiment (n = 13), negative and positive contrast effects were observed. Figure 2A shows an example of a representative NAc neuron that exhibited contrast. This neuron showed a marked reduction in firing rate in response to brief intraoral infusions of the low concentration of sucrose during the continuous phase (Figure 2A, top left PEH). The magnitude of the response was attenuated during intraoral infusions of the same low concentration of sucrose during the alternating phase (Figure 2A, bottom left PEH). The negative contrast effect exhibited by this neuron is characterized with S:B ratios. During the continuous phase the S:B was 0.43, well below 1. During the alternating phase, the S:B ratio was 0.79, approaching 1. This same neuron also showed an inhibition in firing rate in response to brief intraoral infusions of the high concentration of sucrose during the continuous phase (Figure 2A, top right PEH) and the inhibition to this same high concentration of sucrose was of a greater magnitude during the alternating phase (Figure 2A, bottom right PEH). This was reflected in a lower S:B ratio during the alternating versus continuous phases for the high concentration of sucrose. It is important to note that negative contrast attenuated phasic activity while positive contrast augmented the magnitude of the response.

These findings are summarized for the population of neurons that displayed inhibitory responses to the low concentration of sucrose during the continuous phase in Figure 2B. Averaged S:B ratios are presented across all phases of the test session. Post hoc tests of a significant concentration x phase interaction, $F(1, 12) = 25.86$, $p < 0.01$, indicated that the phasic responses of these neurons were attenuated by alternation testing (reflected in higher S:B ratios). These same neurons exhibited a significantly augmented inhibitory response (lower S:B ratios) to the high concentration of sucrose during alternation relative to continuous testing. Thus, these changes reflected neural encoding of negative and positive contrast.

Neurons (n = 13) that exhibited excitatory responses to the low concentration of sucrose during the continuous phase also displayed contrast effects. Figure 2C shows an example of one such representative NAc neuron. This neuron exhibited a marked excitation in firing rate during intraoral infusions of the low concentration of sucrose during the continuous phase (Figure 2C, top left PEH). The response to the same concentration of sucrose was completely absent during the alternating phase (Figure 2C, bottom left PEH). The negative contrast effect was characterized using S:B ratio which was high during the continuous phase but much lower and close to 1 during the alternating phase. This same neuron also exhibited an excitation in firing rate in response to the high concentration of sucrose during the continuous phase (Figure 2C, top right PEH). In addition, the magnitude of the response was more pronounced to this same high concentration of sucrose during the alternating phase (Figure 2C, bottom right PEH). The positive contrast effect was reflected in a slightly higher S:B ratio during the alternating phase.

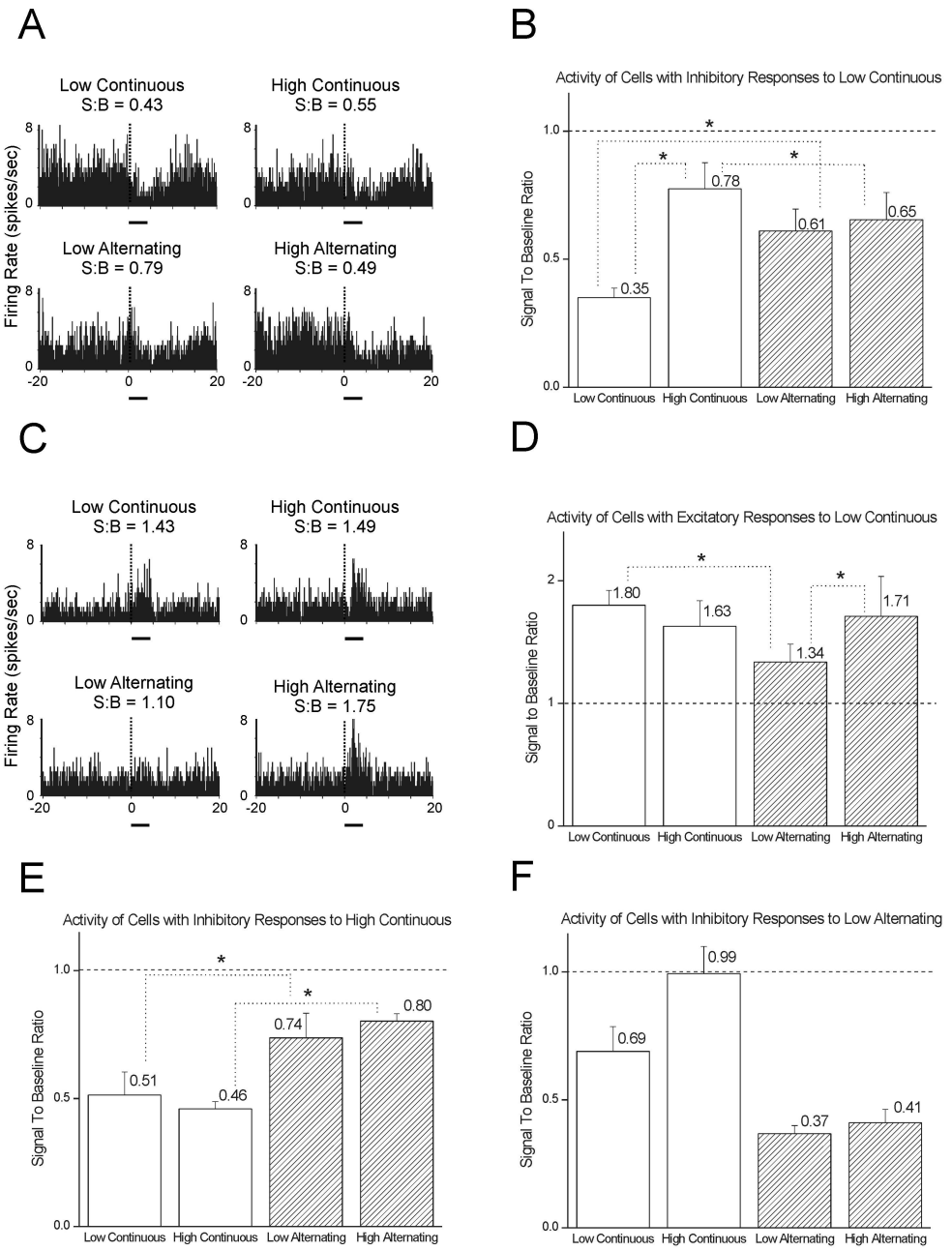

Figure 2. Individual and mean (population) responses reflecting neural encoding of contrast. A. PEHs of a representative inhibitory neuron displaying negative and positive contrast effects. Horizontal bars indicate sucrose delivery. B. Mean (±SEM) S:B ratios across all inhibitory neurons show that the population encodes negative and positive contrast. C. PEHs of a representative excitatory neuron reflecting negative and positive contrast effects. Horizontal bars indicate sucrose delivery. D. Mean (±SEM) S:B ratios across all excitatory neurons show that the population encodes negative contrast. E & F. Populations of neurons showing general sensitivity to contrast testing without clear contrast effects. Each mean S:B ratio is presented above each bar. Asterisks indicate significant differences, $p < 0.05$.

These findings are summarized for the population of neurons that displayed excitatory responses to the low concentration of sucrose during the continuous phase in Figure 2D. Averaged S:B ratios are presented across all phases of the test session. For these neurons, post hoc tests of a significant concentration x phase interaction, $F(1, 12) = 9.31$, $p < 0.05$, showed that responses to the low concentration were attenuated (i.e., S:B ratio was reduced) by alternation testing–reflective of simultaneous negative contrast. Although there was a trend toward a corresponding elevated response for the high concentration in alternation testing above continuous levels, there was not a significant elevation for the population.

Pattern of Stimulus Delivery

Although some NAc neurons exhibited negative and positive contrast encoding, other neurons were sensitive to contrast testing in general. That is, negative and positive contrast testing had the same effect on firing rate. This effect is illustrated for two populations of neurons in Figures 2E and 2F. The neurons shown in Figure 2E ($n = 13$) were classified by their inhibitory responses to the high sucrose concentration during the continuous phase. Interestingly, these neurons had similar inhibitory responses to the low concentration during the continuous phase. Post hoc tests of a significant Concentration x Phase interaction, $F(1, 12) = 9.39$, $p < 0.01$, indicated that inhibitory responses to the low and high sucrose concentrations were similarly attenuated in the alternating phase. While this population of neurons was selective for the continuous testing phase, a second set of neurons was selective for the alternating phase. This population ($n = 11$) was classified by having inhibitory responses to the low concentration during the alternating phase (Figure 2F). A significant main effect of Phase, $F(1, 12) = 11.82$, $p < 0.01$, showed that neurons that were inhibited by the low concentration during the alternating phase also were inhibited by the high concentration during the alternating phase. These neurons were less responsive during the continuous phase to both the low and high concentrations (Figure 2F).

Concentration Effects

Another population of neurons (classified as having excitatory responses to the high concentration in the continuous phase, $n = 11$) failed to show contrast effects but did show concentration selectivity (i.e., this population was responsive to the high but not the low concentration of sucrose, data not shown). This was statistically verified by a significant main effect of Concentration on NAc firing rates for these neurons, $F(1, 10) = 9.70$, $p < 0.05$. In addition, the encoding of sucrose concentration was observed in two previously discussed populations. The population that exhibited inhibitory responses to the low concentration of sucrose during the continuous phase also encoded concentration (Figure 2B). Post hoc tests of a significant concentration x phase interaction, $F(1, 12) = 25.86$, $p < 0.01$, indicated that these neurons were responsive to the low concentration but not the high concentration of sucrose during continuous delivery. The encoding of sucrose concentration also was observed in neurons that displayed excitatory responses to the low concen-

tration of sucrose during the continuous phase (Figure 2D). Post hoc tests of a significant Concentration x Phase interaction, $F(1, 12) = 9.31$, $p < 0.05$, indicated that these neurons were responsive to the high concentration but not the low concentration of sucrose during alternating delivery. This effect emerged during the alternating phase and closely parallels the alteration in oromotor behavior illustrated in Figure 1.

Discussion

Simultaneous contrast as described by Flaherty (1999) has tremendous utility as it guides behavior toward the most valuable, currently available reward by inflating the relative value of the greater reward, and deflating the value of the alternative. Here, rats altered consummatory (oromotor) behavior for intraorally infused sucrose solutions depending on whether they were presented alone or in alternation with another concentration. Consummatory behavior, measured as contractions of the anterior digastric muscle, was reduced for a low concentration of sucrose when it was paired with a higher, more palatable, concentration. That is, negative contrast was observed. In addition, increased consummatory behavior was observed for a high concentration of sucrose when it was paired with a lower concentration. That is, positive contrast was observed. These behavioral results replicate earlier work (Grigson et al., 1997), and were extended here to include an analysis of NAc neuronal firing rates during the expression of simultaneous negative and positive contrast.

Individual NAc neurons modulated their firing rates in response to brief intraoral infusions of different concentrations of sucrose. Interestingly, neural responses to a given concentration of sucrose depended on whether the solution was presented alone or in alternation with a different concentration of sucrose. That is, distinct populations of NAc neurons displayed contrast effects. Specifically, NAc neurons reflected negative contrast where the magnitude of firing rate modulation was reduced for the low concentration when it was presented in alternation with the high concentration of sucrose relative to when it was presented alone. Likewise, NAc neurons reflected positive contrast where the magnitude of firing rate modulation was greater for the high concentration of sucrose when it was presented in alternation with the lower concentration of sucrose relative to when it was presented alone. Other NAc neurons were modulated during alternating sucrose presentations (contrast testing) but not in a directional manner. It is likely that these responses do not reflect contrast effects per se, but rather reflect the encoding of environmental changes independent of reward comparison. Finally, other NAc neurons did not display any neural correlate of contrast behavior but instead displayed greater phasic activity for the higher concentration of sucrose thereby reflecting concentration effects.

The NAc is involved in processing the rewarding properties of palatable foods (Kelley et al., 2002; Kelley, Bless, & Swanson, 1996; Pecina & Berridge, 2000; Zhang & Kelley, 2002). It has been repeatedly demonstrated that individual NAc neurons modulate their firing rate in response to the taste of sucrose (Roitman et al., 2005) as well as while rats are working for sucrose reward (Carelli et al., 2000; Janak, Chen, & Caulder, 2004; Nicola, Yun, Wakabayashi, & Fields,

2004a). However, in these studies, the relative rewarding value of sucrose was constant. Here, the relative reward value of both sucrose solutions was systematically altered using the simultaneous contrast paradigm, as evidenced by our EMG findings, see Figure 1. This led to very different responses from the same NAc neurons to sucrose concentrations when they were presented in isolation or when relative reward value was modified by alternation testing. Recent work by Taha and Fields showed that a subset of NAc neurons with excitatory responses to sucrose exhibited attenuated responses to a low concentration when it was alternated with a higher concentration (Taha & Fields, 2005). We found that inhibitory responses to sucrose also are modulated by contrast testing. In addition is it clear that NAc neurons encode both negative and positive contrast. Further, these effects were demonstrated in a design that removed appetitive aspects of the behavior entirely (i.e., approach), allowing us to focus exclusively on consummatory behavior.

It has been shown that chronically decerebrated rats exhibit simultaneous negative contrast suggesting that the brainstem is sufficient for the processing of behavioral contrast (Grigson et al., 1997). In addition, a more recent investigation has shown that NAc lesions leave consummatory contrast behaviors intact while disrupting the appetitive, goal-directed behaviors in a runway (Leszczuk & Flaherty, 2000). In this light, the observation of contrast responses in the NAc is surprising. While not likely required for the behavioral phenomenon, recent data provide evidence that the NAc tracks both absolute and relative reward properties. The results from microdialysis studies show that NAc dopamine increases with increasing concentrations of sucrose (Hajnal, Smith, & Norgren, 2004), but the dopamine peak to a low concentration of sucrose or saccharin can be blunted when compared with a preferred sucrose reward (Genn, Ahn, & Phillips, 2004) or a drug of abuse (Grigson, Acharya, & Hajnal, 2004). Since the NAc is not necessary for the expression of behavioral contrast, that individual NAc neurons encode contrast may have important implications for the consolidation of these salient reward comparison events for future use. The NAc then, tracks both absolute and relative reward properties, presumably to impact upon subsequent response output. Further investigations of the nuclei and neurotransmitter systems involved in contrast behaviors may ultimately shed light on the neural basis of relative reward encoding and its impact upon behavior.

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