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Experience-dependent changes in neuronal processing in the nucleus accumbens shell in a discriminative learning task in differentially housed rats

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Abstract

Environmental enrichment is associated with enhanced learning of complex tasks, attenuated seeking of natural and drug rewards, and altered function of the nucleus accumbens (NAcc), a brain region involved in goal-directed behavior. For example, during acquisition of a discriminative learning task, neurons in the NAcc core subregion are more responsive to discrete, goal-directed movements in rats raised in an enriched condition (EC) relative to an isolated condition (IC), but as learning materialized, this enhanced responsiveness shifts to the cues that predict these movements. Here, we report that these results do not extend to NAcc shell: neuronal responses in this subregion are similar in EC and IC rats during goal-directed movement and the presentation of associative cues both during and after task acquisition. With experience in this task, however, the overall proportion of task-related neuronal responses in NAcc shell decreases. The response pattern of shell neurons is also sensitive to the presence of contextual cues: shell neuronal firing reveals a significant shift from a predominant excitatory to a predominant inhibitory profile in probe trials when the cue that predicts sucrose availability is absent. Collectively, these data suggest that NAcc shell neurons encode cues associated with natural reward, are less responsive during appetitive behavior in familiar conditions, and are insensitive to appetitive learning differences expressed in rats reared in different environmental conditions.

Keywords

Nucleus accumbens; Environmental enrichment; Social isolation; Single-unit activity; Appetitive conditioning

1. Introduction

Repeated administration of drugs of abuse alter the structure (Robinson and Kolb, 1997) and function (Robinson et al., 1988; White et al., 1995) of the nucleus accumbens (NAcc), a

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brain region known for translating emotional-motivational impulses into goal-directed behavior (Mogenson et al., 1980). These changes are proposed to underlie the transformation of recreational drug use to compulsive drug consumption (Robinson and Berridge, 1993; Robinson and Berridge, 2000). Interestingly, exposure to differential environments during rearing alters reward-seeking behavior (Bardo et al., 2001; van der Harst et al., 2003; Wood et al., 2006) and structure and function of the NAcc (Bowling et al., 1993; Wood et al., 2005). A parallel literature suggests that specialized functional roles exist within NAcc subregions, which closely parallel either striatal or limbic processes: the striatal-related core is preferentially involved in motor control and the acquisition of appetitive learning responses, while the limbic-related shell is highly sensitive to novelty and more closely associated with motivational and visceral mechanisms (Carlezon and Wise, 1996; Maldonado-Irizarry and Kelley, 1995; Maldonado-Irizarry et al., 1995; Rebec et al., 1997a; Rebec et al., 1997b; Sunsay and Rebec, 2008).

Relatively little is known, however, about the impact of differential environmental experience on neuronal processing in the NAcc during goal-directed behavior. In our initial investigation of this issue, we recorded NAcc core neurons of rats reared in an enriched condition (EC) or isolated condition (IC) during acquisition and post-acquisition trials in a discriminative learning task (Wood and Rebec, 2009). At first, these neurons were more responsive to discrete, goal-directed movements in EC rats, but as learning materialized, the neuronal response shifted to the cues that predict these movements. In contrast, these neuronal response patterns in EC and IC animals vanished during post-acquisition trials. Thus, environmental experience alters core neuronal processing of both motor- and sensory-related events during but at different stages during task acquisition. To evaluate the impact of environmental experience on the function of NAcc shell, we recorded the activity of shell neurons in EC and IC rats during acquisition and post-acquisition trials in our discriminative learning task. To assess the importance of the social component of the EC experience, we also characterized the neurobehavioral profile of rats exposed to a social condition (SC) without additional environmental enrichment.

2. Results

Operant Performance

As described elsewhere (Wood et al., 2006), rats were trained in a three-phase shaping procedure for sucrose reinforcement culminating in a discriminative learning task. In phase one, animals were introduced to a spout that released sucrose. This availability temporally corresponded with presentation of a feeder cue. In phase two, nose pokes were required to elicit the feeder cue (sucrose availability). In the third and final phase of training, a discriminative nose poke to one of two nosepoke holes (correct response) was required to elicit the feeder cue. As indicated by a higher proportion of correct responses, EC rats ($n = 21$) acquired this discriminative behavioral response (phase-three training) more rapidly than IC rats ($n = 17$) [$F(2,46) = 4.571$, $p = .015$, see Fig. 1]. The performance of SC rats ($n = 11$) was highly variable and thus were not significantly different from either EC or IC rats ($p = \text{ns}$). As indicated, EC-IC performance differences were evident by the third training session, while behavioral performance during initial training and post-acquisition recordings were similar in all three groups.

Electrophysiology

During task acquisition (first and third training sessions) and post-acquisition sessions, we recorded a total of 418 neurons in NAcc shell of EC, IC and SC rats (see Fig. 2 for placement of these electrode bundles). The number of shell neurons ($n = 133\text{--}149$) recorded during each session was roughly comparable, while 26–57 neurons were recorded from each

group in each recording session (see Table 1). Spike waveforms ranged between 0.6 and 1.2 ms in duration and 300 and 500 μ V in amplitude. Signal-to-noise ratios of discriminated waveforms typically ranged between 3 and 4:1. Mean baseline firing rates of shell neurons in these three groups were not significantly different [$F(2, 415)=1.086$, $p=0.339$; see Table 1], roughly 2 imp/sec in each case. Peri-event histograms (PEHs) were used to evaluate neuronal responses during the following task events: nose-poke cue, correct response (operant response), and feeder cue (see Data Analyses). Examples of task-related feeder cue responses recorded from different shell neurons in an EC rat, IC rat and SC rat are provided in Fig. 3.

During the first training session, a substantial proportion (~30-50%) of neurons exhibited phasic firing changes during all three task events in all three groups. The proportion of neuronal responses in EC, IC, and SC animals was also similar during these task events in the third training session, with roughly 25-35% of neurons responding during each event. A similar profile emerged during post-acquisition recordings. In fact, there were no significant group differences during these events in any one of these three recording sessions (see Fig. 4). The magnitude of neuronal responses during each task event in each recording session was also comparable across groups: mean excitations ranged between 160 and 377% and inhibitions between 52 and 90% of pre-event baseline.

In all groups, moreover, the overall proportion of task-related shell neuronal responses during these appetitive task events showed a consistent effect between recording sessions. As indicated in Fig. 5, the proportion of responsive shell neurons decreased from the first training session to post-acquisition sessions for nose-poke cue ($X^2=4.57$; $p<.05$), correct response ($X^2=5.23$; $p<.05$), and feeder cue ($X^2=9.80$; $p<.05$). In fact, this decrease in overall responsiveness was evident from the first to the third training session in two of the task events: correct response ($X^2=4.04$; $p<.05$) and feeder cue ($X^2=7.40$; $p<.05$).

To assess the dependence of neuronal firing changes on task-related stimuli during task acquisition, probe trials, in which a particular stimulus was removed from the trial (see Experimental Procedure), were inserted in a subset of recordings from EC ($n=1$), IC ($n=1$), and SC ($n=6$) rats. Although the neuronal response pattern was not altered by nose-poke cue probe trials, a significant change in the profile of the responses of shell neurons ($n=40$, collected from all three groups) occurred during feeder-cue probe trials. In standard trials, 13 of the 18 responsive neurons increased firing rate during feeder cue. A very different response appeared in feeder-cue probe trials (see Fig. 6). Fewer responses occurred in the absence of the cue (14/40); more interestingly, the predominant response pattern shifted towards an inhibitory response (11/14). This altered response profile was significant ($X^2=8.13$; $p<0.01$) due mainly to a loss of excitatory responding during feeder cue-probe trials. Interestingly, the behavioral profile expressed during the feeder-cue probe trials was similar to that expressed during standard phase three trials: 88% of the probe trials elicited correct responses, while 87% of those were reinforced.

3. Discussion

During acquisition but not post-acquisition recordings, IC rats learned the operant response for sucrose reinforcement slower than EC rats. Interestingly, there were no group differences in the response of shell neurons to the assessed appetitive task events (nose-poke cue, correct response, and feeder cue) during acquisition or post-acquisition recordings. The observed significant changes in NAcc shell neuronal responding, however, occurred independent of housing experience. For example, shell neurons overall were significantly less responsive during nose-poke cue in post-acquisition recordings relative to the first training session. Similar patterns were evident during correct response and feeder cue: the

proportion of responsive shell neurons progressively decreased during these task events through subsequent exposure to the phase-three protocol. The reduced responsiveness of shell neurons during correct response and feeder cue was significant by the third training session.

An influential hypothesis of NAcc function implicates the NAcc in the regulation of flexible, approach behavior leading to the formation of incentive representations with reinforcing stimuli (Ikemoto and Pankseep, 1999). As animals obtained phase-three training in our discriminative learning task, their goal-directed behavior was generally less variable and more discriminative as indicated by an acceleration of trial completion rate and increased nose-poke accuracy. Based upon the flexible approach hypothesis, neuronal responsiveness in NAcc should decrease as animals acquire the phase-three protocol. Our report of task-related neuronal responses in the NAcc core of differentially housed animals during the acquisition of this appetitive task is inconsistent with this hypothesis (Wood and Rebec, 2009). The proportion of task-related responses of core neurons in IC rats was generally low in initial phase-three training but consistently increased with training. The responsiveness of core neurons in EC rats was usually high during each assessed task event in initial training. As these animals improved their performance, however, the proportion of task-related responses generally remained high and in some cases (e.g. during feeder cue) exceeded the proportion of responses in the first training session.

In contrast, the task-related neuronal responses from NAcc shell that we report here are supportive of the flexible, approach hypothesis. During all three appetitive task events (nose-poke cue, correct response, and feeder cue), the overall proportion of responsive neurons decreased. These results are consistent with the notion that NAcc shell is involved in regulating flexible, approach behavior prior to contextual familiarity. Certainly, after the completion of phase-two training, all rats experienced novel circumstances under the phase-three protocol (see Experimental Procedure). As the new contingencies of the phase-three protocol became more familiar and/or less novel, shell neurons became less responsive during these appetitive task events. Previous data collected from our laboratory suggest that NAcc shell is involved in regulating appetitive behavior expressed in novel conditions. Following entry into a novel environment, dopamine efflux increased in NAcc shell but not NAcc core (Rebec et al., 1997a; Rebec et al., 1997b). In fact, neuronal recordings in this paradigm indicate that during approach to novelty, the response pattern in core and shell neurons differed (Wood and Rebec, 2004), further indicating that these subregions function differently when appetitive behaviors are expressed in novel conditions.

Given that NAcc shell plays a role in novelty detection and that environmental enrichment involves repeated novelty exposure, one might expect that shell function would be altered in EC relative to IC rats during learning of the nose-poke reinforcement task. Our analysis, however, did not confirm this. Because EC rats locomote less and habituate more quickly to a novel environment, evaluation of neuronal responses when animals were first introduced to the paradigm (in phase 1 training) may have been a more appropriate point in training to assess environmental influences on shell neuronal responses. We focused instead on phase-three training because of the clear behavioral differences that emerged during this phase. In contrast to the lack of observed neuronal response differences in NAcc shell, several differences were evident in the activity of NAcc core neurons of EC and IC rats during acquisition of the nose-poke reinforcement task (Wood et al. 2009). Interestingly, pharmacological blockade of NMDA receptors in core but not shell disrupts acquisition of an appetitive learning task (Kelley et al., 1997), suggesting that neuronal activation in the NAcc core may explain the differential acquisition rate of EC and IC rats in our paradigm. Consistent with this possibility, NMDA receptor subunit expression in NAcc core is altered by differential housing experience (Wood et al., 2005).

Converging anatomical and electrophysiological data indicate that subicular and CA1 regions of the hippocampus may underlie novelty-related activation of shell neurons. Glutamatergic afferents from these brain areas preferentially target the shell subregion (Brog et al., 1993; Groenewegen et al., 1987), while electrophysiological recordings in CA1 and subiculum reveal robust phasic activation during orientation to novel but not familiar stimuli (Vinogradova, 2001). In addition, ventral hippocampal lesions abolish exploration of a novel object (Mellanby et al., 1999). Furthermore, electrical stimulation of hippocampus results in robust NAcc neuronal activation (O'Donnell and Grace, 1995). Collectively, these results suggest that glutamatergic inputs from these hippocampal regions may participate in the activation of shell neurons when appetitive behavior is expressed under novel conditions.

We used SC rats, in part, to determine if their neurobehavioral profile more closely paralleled that obtained from EC or IC rats. Other studies have used SC rats as a control group for both EC and IC conditions and demonstrated related experience-dependent changes. For example, EC relative to SC rats show reduced anticipatory responding to a conditioned stimulus (CS) signaling sucrose reward (van der Harst et al., 2003). Likewise, IC rats exhibit elevated approach responding to a CS signaling food reward (Harmer and Phillips, 1998; Morgan and Einon, 1975; Scott et al., 1990), a higher propensity to self-administer drugs of abuse (Alexander et al., 1978; Schenk et al., 1987), and response-inhibition deficits (Dalrymple-Alford and Benton, 1984; Morgan and Einon, 1975; Morgan et al., 1977) relative to SC controls. Collectively, these findings indicate that both the social and environmental context affect appetitive behavior in EC rats. In the present study, however, the nose-poke accuracy of SC rats was not significantly different from either EC or IC rats during acquisition of our appetitive learning task. Similarly, the responsiveness of shell neurons in SC rats like those recorded in EC and IC rats decreased during all three appetitive task events from the first training session to the post-acquisition sessions.

Probe trials were used in a select number of cases to assess the dependence of neuronal changes and task-related stimuli. Although no consistent change was observed in the firing patterns in nose-poke-cue probe trials, there was a significant change in the composition of shell responses during feeder-cue probe trials. In these trials, most shell excitations that were present during the standard protocol were either not evident or were replaced by inhibitions. Since the overt movement expressed during these task events in both the standard protocol and these probe trials was similar (in the absence of the feeder cue, animals continued to collect their reinforcement), these findings suggest that the increased firing that occurred coincident with this task event during the standard protocol were associated with the sensory expectancy of these cues.

Consistent with these data, shell neurons exhibited significantly greater responses to a discriminative stimulus during extinction from cocaine self-administration, while core neurons responded indiscriminately to both the discriminative stimulus and different neutral stimulus (Ghitza et al., 2003). In other work involving cocaine self-administration, core neurons showed greater phasic firing changes than shell neurons preceding reinforced and non-reinforced lever-presses (Ghitza et al., 2004). Collectively, these data may indicate that shell neurons are involved in discriminating the motivational value of stimuli known to be associated with reward, while core neurons may be preferentially involved in the regulation of operant responding and other learned reward-seeking behavioral responses.

Clearly the NAcc is critically involved in feeding and drug-taking as well as aspects of learning that relate to both types of reward-seeking responses. Moreover, study of NAcc core and shell reveal functional differences in appetitive learning tasks, although relatively little is known about the differential neuronal responses expressed in NAcc core and shell during the acquisition of an appetitive task. Because learning is an important aspect of

developing the maladaptive appetitive behavioral responses that underlie drug addiction, understanding the neuronal processes that underlie these phenomena are crucial. Our results reveal that the functional roles of NAcc core and shell are not static in the acquisition of appetitive behavioral responses but rather change differentially by both task and housing experience during the learning process.

4. Experimental procedures

Animal Care

Male rat pups bred from adult Sprague-Dawley rats (Harlan Industries, Indianapolis, IN, USA) were cross-fostered to a single mother on post-natal day (p) 2, weaned on p28, and placed into either IC (20 × 24 × 18 cm), EC (43 × 90 × 46 cm), or SC (25 × 43 × 18 cm) housing. EC rats were housed in small groups (n=3-5), SC rats were housed in pairs, and IC rats were housed individually. The EC cage contained toys, tunnels, and other interactive objects, all of which were repositioned three times/week; no group was handled during the experimental housing period. Animals were provided ad libitum access to food and water and maintained on a 12 hr light/dark cycle (07:00 lights on). From p45 to p55, food (but not water) was restricted to maintain animals at 85% of their free-feeding weight with operant training beginning 5 days later. After phase-one and phase-two training (see below), animals returned to free feeding for at least 48 hrs before undergoing surgery. Following recovery from surgery, animals were returned to food restriction. Training resumed when animals returned to 85% of their free-feeding weight (~7-10 days). All animal protocols were approved by the Indiana University Institutional Animal Care and Use Committee.

Training Protocol

Rats were trained in a three-phase shaping procedure for sucrose (10% w/v) reinforcement. In phase one, rats were prompted to lick a spout for sucrose in association with the feeder cue consisting of a tone (4500 Hz, 70 dB) and a yellow LED located above the spout. The yellow LED remained illuminated until the rat broke the photo beam located in front of the spout triggering a 1 s release of sucrose (sucrose consumption) followed by a 5 s intertrial interval (ITI). To facilitate association of the feeder cue and sucrose availability, the ITI was increased by 5 s for every 5 trials thereafter. Following 5 trials with a 30 s ITI, phase-one training was complete.

In phase two, animals were required to poke one of those two lit holes (nose poke) to elicit the feeder cue. A partition that blocked access to two nose-poke holes on the opposing wall of the chamber was removed as the first session of phase two began. An initial, free reinforcement (signaled by a feeder cue) was provided on the first trial. Each subsequent trial began with a lit green LED in both nose-poke holes coincident with a 900 ms nose-poke cue tone (1900 Hz, 70 dB) following a 3–8 s ITI. The green LED remained lit until a nose poke was performed, which was followed by the feeder cue 700 ms later. When the rat broke the photo beam in front of the spout, the feeder cue was deactivated, and sucrose was consumed. Thus, rats walked from the nose-poke side of the chamber to the opposite side to receive reinforcement. To ensure sucrose consumption during the initial trials of the first session of phase two, sucrose was available for 60 s following a nose poke. After the first 10 reinforced responses, this period was reduced to 15 s. When animals performed 30 trials in 30 min, phase two was complete.

In phase three, only one nose-poke hole was lit (pseudorandomly selected) at trial onset to stimulate selective responding. After an initial, free reinforcement, the feeder cue was activated 700 ms after a nose poke to the lit hole (correct response). In the event of an incorrect response, the nose-poke light stayed on until a correct response was made. As was

the case in phase two, the feeder cue was deactivated upon sucrose consumption, which was followed by an inter-trial interval of 3-8 s. After feeder cue onset, sucrose was available for 10 s. Following the first 70 trials, sucrose availability was reduced from 10 to 5 s. Each training session lasted 45 min or 100 trials, whichever occurred first. Phase-three training was considered complete after three training sessions. Subsequent sessions were known as post-acquisition sessions, in which rats expressed at least 80% accuracy (typically 20 or fewer bad pokes in a 100-trial session). Bad pokes were defined as nose pokes to the incorrect hole after nose-poke cue onset prior to a correct response.

A limited number of recordings (n=8) included probe trials. Because we felt that an equal number of probe and standard trials should be analyzed to make the claim that responsiveness disappeared, each rat that presented with probe trials first received 50 standard trials, which were immediately followed by 50 trials of nose-poke cue and feeder cue probe trials respectively. The probe trials were evaluated separately from the standard trials.

Surgical Procedure

Rats were implanted with microwire electrode bundles to record single unit activity from NAcc shell. When each animal was fully anesthetized (90 mg/kg ketamine HCl; 10 mg/kg xylazine HCl; im) and secured in a stereotaxic frame, the skull was exposed, and holes were drilled bilaterally over NAcc shell (0.8 mm lateral and 1.7 mm anterior to bregma) according to standard coordinates (Paxinos and Watson, 1998). A cylindrical bundle of eight microwire electrodes (Teflon-coated, 50 μ diameter, stainless-steel) and one ground wire soldered to a 10-pin connector (NB Labs, Denison, Texas), were lowered to the proper depth (6.5-7.5 mm ventral from brain surface). Six stainless-steel support screws were attached to the skull along with dental acrylic to stabilize neuronal recordings. At the conclusion of surgery, each rat was injected with Ringer's solution (5-10 ml, sc) to counter dehydration.

Electrophysiology

A flexible, shielded cable was attached to the connector of each implanted electrode bundle and plugged into the commutator located above the operant chamber. Partitions were placed in the operant chamber to impede access to operant stimuli while neuronal activity was assessed. Neuronal signals from the rat were amplified, filtered (bandpass, 0.3-10 kHz), and routed to a Multi-channel Neuronal Acquisition Processor (MNAP, Plexon, Inc., Dallas, TX) for spike discrimination. These signals were also routed to an oscilloscope and audio monitor to facilitate the discrimination of single units. Time stamps for each unit were confined to those waveforms that selectively passed through a closely matched, digitized waveform template.

Waveforms of different units recorded on the same microwire were separated on the basis of their distinct characteristics including amplitude, duration, and voltages at specific points on the ascending and descending limbs of each respective waveform. Waveforms with a consistent amplitude and signal-to-noise ratio (at least 2.5 to 1) were identified as single units; this characterization was later confirmed by ad hoc analysis of autocorrelogram data. After unit discrimination, the partitions within the operant chamber were removed, and the first session of phase-three training or the post-acquisition session began. Behavioral protocols were controlled and monitored by a computer that interacted with the operant chamber and MNAP via a software interface (Q-UNIT) that sent digital pulses to the MNAP system corresponding to behavioral output. MNAP simultaneously tracked operant events along with the collection of electrophysiological data.

Tissue Preparation

After the last recording session, rats were overdosed with a mixture of pentobarbital and chloral hydrate (chloropent; 0.33 ml/kg; i.p.) and current (30 μ A, 5 s) was passed through the electrodes to mark recording sites. Rats were perfused transcardially with an isotonic saline followed by a solution of 4% paraformaldehyde and 5% potassium ferrocyanide. Metal deposits left at each lesion site reacted with this solution to produce a localized blue-green stain (Prussian Blue reaction). Brains were removed, fixed in 10% formalin, and placed in a 20% sucrose solution until they were sectioned. Tissue was cut in 40 μ m coronal sections, stained with Cresyl Violet, and examined for the location of each electrode bundle.

Data Analyses

Changes in spike activity of NAcc shell neurons were assessed via peri-event histograms (PEHs) during three task events (nose-poke cue, correct operant response, and feeder cue) in the first and third sessions of phase-three training and the post-acquisition session. Based upon a pre-event baseline (mean firing during the 300 ms period immediately before event onset), neuronal excitations and inhibitions occurred for each task event if the firing rate in three, consecutive 50-ms bins during the 300 ms immediately after event onset increased or decreased by at least 30%. These criteria were established to detect changes beyond normal fluctuations in baseline activity and are consistent with established standards used to identify responses in NAcc neurons during appetitive behaviors (Carelli and Deadwyler, 1994). Analysis of variance and t-tests were used to assess group baseline firing rates (preceding each trial), neuronal response magnitudes, and nose-poke accuracy defined as percentage of correct operant responses/session [number of correct responses/total responses (correct responses + bad pokes)/session]. Pearson's chi-square tests were used to compare the proportion of task-related responsive units for each task event for the phase-one, phase-three and post-acquisition recording sessions.

To assess the dependence of neuronal firing changes and task-related stimuli during task acquisition, several recordings incorporated probe trials in which the placement of the stimulus was removed. In nose-poke cue probe trials, there was no ITI period preceding nose-poke cue after sucrose reinforcement. In feeder-cue probe trials, sucrose was made available after a correct response although the feeder cue was not provided. PEHs were used to assess neuronal responses during the probe trials in the same manner as in unaltered trials.

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Fig. 1. Percent correct responses/session in EC, IC, and SC rats during the first and third session of phase three training and during the post-acquisition session. As indicated (*), EC rats exhibited significantly greater nose-poke accuracy than IC rats during the third session (3×; $p < .05$); no group differences were observed during the first (1×) or post-acquisition (PA) session.

Fig. 2.

Placement of microwire electrode bundles in the left and right NAcc shell in EC (left series; n=16), IC (center series; n=11), and SC (right series; n=11) rats. As indicated by black dots, the locations of the tip of these bundles were found along the rostral-caudal axis (2.7-1.2 mm anterior to bregma) of NAcc shell.

Fig. 3. Peri-event histograms of single-unit responses (excitations) recorded from shell neurons in an EC rat (A), IC rat (B), and an SC rat (C) during feeder cue. Onset of feeder cue occurred at time 0.

Fig. 4. Percentage of total responsive units (excitations and inhibitions) in NAcc shell during nose-poke cue (NPC), correct response (CR), and feeder cue (FC) in EC, IC, and SC rats organized by recording session. Note that no group differences were apparent during nose-poke cue, correct response, and feeder cue in the first, third, or post-acquisition session.

Fig. 5.

Proportion of shell neurons during (A) nose-poke cue (NPC), (B) correct response (CR), and (C) feeder cue (FC) in the first, third, or post-acquisition session. As indicated (*), the decreased responsiveness of shell neurons from the first to the third training session during CR and FC was significant ($p < 0.05$). In addition, decreased responsiveness from the first training session to the post-acquisition session ($p < 0.05$) was significant for all three of these appetitive task events.

Fig. 6.

Peri-event histogram for two neurons recorded in the first session of phase-three training. Units A and B were recorded under the standard protocol (A1, B1) and during feeder-cue probe trials (A2, B2). Correct response occurred at time 0 and feeder cue under the standard protocol occurred 700 ms later (triangles mark feeder cue onset in A-1 and B-1). During feeder cue-probe trials, the feeder cue is absent. Note that firing rate increased immediately after feeder-cue onset under the standard protocol (A1, B1), but was absent during the probe trials (A2, B2).

Table 1

Total number of EC, IC, and SC animals in the study, the number of EC, IC, and SC animals in which shell neurons were recorded (Shell recordings), and overall mean firing rate of shell neurons recorded from each group (Overall mean firing).

Recording	EC	IC	SC
Total animal number	21	17	11
Shell recordings	16	11	11
Overall mean firing (Imp/s; $\pm$ S.E.M)	2.21($\pm$.23)	2.07 ($\pm$.25)	1.70 ($\pm$.15)

Table 2

Total number of EC, IC, and SC neurons recorded in the first training session, the third training session, and the post-acquisition session.

Recording	EC	IC	SC	Overall
1 st session	57	44	48	149
3 rd session	41	46	49	136
post-acquisition	57	50	26	133