Nucleus Accumbens Neurons Track Behavioral Preferences and Reward Outcomes During Risky Decision Making

Supplemental Information

Supplemental Methods

Animals

Male Sprague Dawley rats (*n* = 17, Harlan Sprague Dawley, Indianapolis, IN), aged 90-120 d and weighing 275-350 g were used as subjects and individually housed with a 12/12-h light/dark cycle. All experiments were conducted between 8:00 am and 5:00 pm. Animals were maintained at no less than 85% of pre-experimental bodyweights by food restriction (~10-15 g of Purina laboratory chow each day in addition to approximately 1 g of sucrose consumed during behavioral sessions) except during the post-operative recovery period when food was given *ad libitum*. All procedures were approved by the University of North Carolina Institutional Animal Care and Use Committee.

Surgery

Prior to the start of behavioral training, rats underwent surgery for implantation of microelectrode recording arrays into the nucleus accumbens (NAc) core and shell. Surgery was conducted under anesthesia with ketamine (100 mg/kg) and xylazine (20 mg/kg) using established procedures routinely used in the Carelli laboratory (1-4). Custom designed electrode arrays (NB Labs, Denison, TX) were stereotaxically guided into the core (AP: +1.7; ML: +/- 1.3 relative to bregma; DV: -6.5 relative to skull surface) and shell (AP: +1.7; ML +/- 0.8 relative to bregma; DV:-6.5 relative to skull surface) of the NAc. Each array consisted of eight microwires (50 μm diameter) arranged in a 2 X 4 bundle that measure ~1.5 mm anteroposterior and ~0.75 mm mediolateral. Ground wires for each array were inserted into the brain remote to electrode

arrays. The arrays and ground wires were anchored to the skull via stainless steel screws and dental acrylic. All animals were allowed at least five post-operative recovery days before beginning training on the behavioral task.

Behavioral Training

Following recovery from surgery, rats were trained on a risk-based decision making task developed in our laboratory (5). Rats received at least 25 training sessions on the task prior to electrophysiology recordings. All training was conducted in 43 X 43 X 53 cm Plexiglas chambers housed in sound-attenuated cubicles (Med Associates, St Albans, VT). One side of the chamber had 2 retractable levers (Coulbourn Instruments, Allentown, PA) 17 cm apart, with a stimulus light 6 cm above each lever. A white noise speaker (80 db) was located 12 cm above the floor on the opposite wall. A houselight (100 mA) was mounted 6 cm above the speaker. Sucrose pellets (45 mg) were delivered to the food receptacle located equidistantly between the levers. Initially, lever pressing behavior in all rats was reinforced on a continuous schedule of reinforcement on two levers, such that every response on either lever resulted in the delivery of a 45 mg sucrose pellet to a centrally located food receptacle. Rats could make 50 presses on each lever for a maximum of 100 reinforcers per session. Once rats reached stable responding (50 presses on each lever; 5 training sessions) daily training began on the risk-based decision making task in which rewards were contingent on operant responses in 90 discrete trials per session. Importantly, each trial was initiated randomly after a variable time interval with an average of 30 seconds between trials. Distinct cue lights were illuminated (5 s) before lever extension and levers were available for 15 s unless the response requirements were completed. Upon completion of the appropriate requirement the lever was retracted and behavior was rewarded. There were 60 forced trials in which one cue light was presented alone and a response on the

corresponding lever was reinforced with 45 mg sucrose pellets. Responses on the non-cued lever were counted as "errors" (houselight extinguished and no reward delivered). The number of errors was used as a behavioral measure of discrimination between the two different response options. For the first 10 training sessions each lever was reinforced on a fixed ratio 1 (FR1) schedule with one sucrose pellet. This was done to allow animals to fully learn the predictive associations of the cue lights before the reward contingencies were altered. Furthermore, this ensured that there would be no bias in response allocation as a result of differential learning between the two levers. For the next 15 sessions one lever (counterbalanced across animals) was designated the "risky lever" and was reinforced on 50% of the trials with 2 sucrose pellets while the other lever, designated the "safe lever" remained on the original contingency of 1 sucrose pellet 100% of the time (Figure 1A). During each behavioral session there were also 30 free choice trials in which both cue lights were illuminated and both levers were active such that the rat was rewarded based on the contingency of reinforcement for the lever chosen. Response allocation on free choice trials was used to determine the subjective value associated with each response option. Animals were considered to have a behavioral preference if they displayed 60% responding for the preferred lever during choice trials. Electrophysiological activity of NAc neurons was recorded during the final behavioral session. A subset of animals (n = 8) did not display a behavioral preference during the first recording session and therefore continued training until a behavioral preference developed (3-8 additional training sessions). Animals were then recorded when a stable behavioral preference developed.

Electrophysiological Recordings

The procedure for extracellular recording in the NAc during behavior is routinely used in the Carelli laboratory and is described in detail elsewhere (3, 4, 6). Briefly, before the start of the

session, the rat was connected to a flexible recording cable attached to a commutator (Crist Instrument Company, Inc.) which allows for virtually unrestrained movement within the chamber. The headstage of each recording cable contains 16 miniature unity-gain field effect transistors. Online isolation and discrimination of neuronal activity was accomplished using commercially available neurophysiological system (MAP System, Plexon, Inc., Dallas, TX). Criteria for identifying different neurons on a single wire have been described in detail elsewhere (7-9). Briefly discrimination of individual waveforms corresponding to a single cell was accomplished using template analysis procedures provided by the neurophysiological software 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 cell. Principal component regression of waveform data was conducted using the Offline Sorter Program (Plexon, Inc) to further separate waveforms recorded from the same microwire.

Neural Analysis

Analysis of neural activity collected during behavioral sessions had two main goals. First, we identified if neurons exhibited increases (excitations) or decreases (inhibitions) in activity relative to the behavioral events. Secondly, we evaluated if the response patterns of neurons were sensitive to differences in risk versus safe options. Cells that showed significant differences (either excitation or inhibition) following the onset of a behavioral event (e.g., cue presentation) were considered phasic for that event. Cells with a baseline firing rate of less than 0.1 Hz (n = 9) were excluded from analysis to ensure the ability to detect both excitations and inhibitions. Cell type classification was determined by performing a 2-way analysis of variance (ANOVA) for each cell using the mean firing rate for each trial in each analysis period similar to previous

reports (10, 11). Cell firing was analyzed over 5 time epochs within each given trial: the 10 s baseline period was compared to the first 2.5 s following cue onset (Cue period), the 2.5 s prior to lever press (Prepress period), and two 2.5 s epochs during the reward period (Early Reward and Late Reward periods) (Figure 2A). In each analysis, a single cell was analyzed with bin as a repeated-measures factor (e.g., baseline bin vs. cue bin), and trial type (e.g., risk vs. safe; reward omission vs. large reward vs. small reward) as an independent-measures factor. We then determined the type of phasic encoding by comparing firing rates during the bins: *phasically modulated* cells showed significantly different activity during the effect period (cue, prepress, reward) compared to baseline. *Nonphasic* cells showed no differences between the effect periods or baseline bins. *Selective* cells were classified as cells that were phasic to only one trial type and/or cells that were phasic to both trial types and showed significantly different firing rates during each of the trial types (e.g., risk vs. safe cue). Further, we evaluated the percentage of all cells that encoded a particular event (e.g., risk cue) on each session and in each region (core or shell). This cell count analysis was conducted separately for neurons in the nonpreferring, risk preferring, and safe preferring groups. Differences in the frequency or proportion of neuronal responses across different trial types, subregions, or reward preferences were examined using chi square analysis.

In order to further examine differences in neural activity for the risk versus safe options, we evaluated how neural populations encoded each of these options relative to each animal's individual preference. For animals that showed a significant preference for one of the options during choice trials (i.e., at least 60% responses for one of the safe or risk option compared to the other), cell firing in the core and shell was normalized to allow population analysis. For this, the average firing for each cell was grouped into 200 ms bins, and the mean firing and standard

deviation was taken for each 10 s baseline. Each bin was then z-normalized by subtracting the average baseline firing rate from the firing rate in each 200 ms bin, divided by the standard deviation. We then grouped phasic cells (i.e., those showing significant excitations or inhibitions relative to an event) according to their behavioral preference, specifically, looking at risk-encoding cells from risk-preferring rats and safe-encoding cells from safe-preferring rats. This comprised the set of Preferred Cells, while the opposite trial option (e.g., safe-encoding cells in risk-preferring rats) were the set of Nonpreferred Cells. For Forced choice trials, these were taken from the Forced Safe and Forced Risk trials. For Free Choice trials, data were separated into Preferred/Nonpreferred by the subsequently made choice after the cue (i.e., they chose their preferred versus their nonpreferred option). This was done separately for Forced Preferred/Nonpreferred and Free Choice Preferred/Nonpreferred so that we could examine whether any differences existed between forced trials (where no "decision" is necessary) and free choice trials, when animals are required to make a decision between competing options.

To best isolate the effects of preference in the core and shell, we separately averaged cells displaying significant excitatory and inhibitory encoding for the preferred option in the core and shell, respectively. To quantify these differences, peak normalized firing rate (greatest firing in excitatory cells; greatest decrease in firing for inhibitory cells) within 2 s of stimulus onset were averaged for all included cells for the preferred and nonpreferred option during forced and choice trials. Peak differences were quantified using a 2-way ANOVA with Preference (Preferred vs NonPreferred stimulus; within-subjects factor) and Trial Type (Forced vs Choice; between subjects factor), and Tukey's HSD as a post-hoc test for pairwise comparisons. In analyses where there were low *n* (6 or fewer cells in a group), a non-parametric Wilcoxon signed-rank test was used to compare the difference in peak firing between the preferred and nonpreferred option. All

analyses were considered significant at $\alpha = 0.05$. Statistical and graphical analyses were conducted in Graphpad Prism 4 (Graphpad software, Inc.) and STATISTICA (StatSoft, Tulsa, OK).

Histology

Upon completion of the last experiment, rats were deeply anesthetized with a ketamine/xylazine mixture (100 mg/kg and 10 mg/kg, respectively). To mark the placement of electrode tips, a 13.5 µA current was passed through each microwire electrode for 5 s. Transcardial perfusions were then performed using physiological saline and a 10% formalin mixture containing potassium ferricyanide (3%), which reveals a blue dot reaction product corresponding to the location of each electrode tip. Brains were then removed, post-fixed using a 10% formalin solution, and frozen. After postfixing and freezing, 30 µm coronal brain sections were mounted on microscope slides. The specific placement of the electrode tips in the NAc core or shell were verified using a standard sterotaxic atlas (12). Neurons recorded on electrodes placed outside of the NAc core or shell were excluded from all analyses.

Supplemental Results

Prepress Population Activity Analysis

As with cue-related encoding, there were marked differences in the NAc core population activity based on the animals' preference during choice behavior, but not forced choice trials. Specifically, press-related excitations in the core (Figure S2A,B) showed no differences in peak firing during forced trials Wilcoxon, W = -2, n.s., but again showed differential firing during free choice trials, Wilcoxon, W = 16, $p = 0.05$ (Figure S2C). Unexpectedly, there was a slightly

greater inhibition seen for the nonpreferred cue than the preferred cue during forced trials (Figure S2D), though during choice behavior, there were greater inhibitions for the preferred cue (Figure S2E). Peak inhibitions in the core revealed a significant interaction between trial type X preference, $F_{(1,47)} = 72.99$, $p < 0.0001$. Specifically, this was due to an increased inhibitory response for the nonpreferred cue compared to the preferred cue during forced trials ($p < 0.05$), and a significantly blunted inhibitory response during the choice cue when animals selected the nonpreferred option compared to the preferred option, $p < 0.0001$ (Figure S2F). As during the cue, this blunted response was significantly less inhibitory than any of the other cue options ($p < 0.001$ vs Forced Preferred, $p < 0.001$ vs Forced Nonpreferred).

In the shell, excitatory responses during the prepress period on forced (Figure S3A) and free choice (Figure S3B) trials were similar, an observation supported when peak firing was examined (Figure S3C). There was a trend towards an interaction between trial type X preference, $F_{(1,25)} = 3.27$, $p = 0.08$, which posthoc tests indicated was due to greater firing during performance of the preferred option during choice trials ($p = 0.035$) but not during forced trials ($p = 0.77$). However, inhibitions in the shell were similar to those seen for the cue; greater inhibitions were seen for the preferred option regardless of trial type. A main effect preference, $F_{(1,64)} = 34.8$, $p < 0.0001$, but no interaction between preference X trial type was found, $F_{(1,64)} = 2.14$, $p = 0.15$. Planned pairwise comparisons showed that there were significantly greater inhibitions to the preferred action compared to the nonpreferred during both forced trials ($p = 0.01$) and choice trials ($p < 0.001$).

Finally we evaluated the population of neurons recruited to encode the forced and choice trials during the prepress period. As during the cue period, neurons that encoded information about the preferred action during forced trials but not choice trials were “Forced Only” and

likewise for choice trials ("Choice Only"). Cells that encoded information about the preferred action in both forced and choice trials were "Both Forced/Choice", which cells that failed to encode either were considered nonphasic. Interestingly, though there were a large percentage of neurons that phasically encoded information about press in the core (39/89 [44%]) and shell (54/132 [41%]), there were no differences between regions for encoding type, $\chi^2_{(2)} = 3.34$, $p = 0.19$. Specifically, there were no differences between core and shell for Forced Only (core: 10/39, shell: 11/54; $\chi^2 = 0.72$, $p = 0.39$), Choice Only (core: 6/39, shell, 4/54; $\chi^2 < 1$, $p = 0.31$), or Both Forced/Choice (core: 22/39, shell: 40/54; $\chi^2 = 2.43$, $p = 0.12$) (Figure S4) as was seen during the cue period.

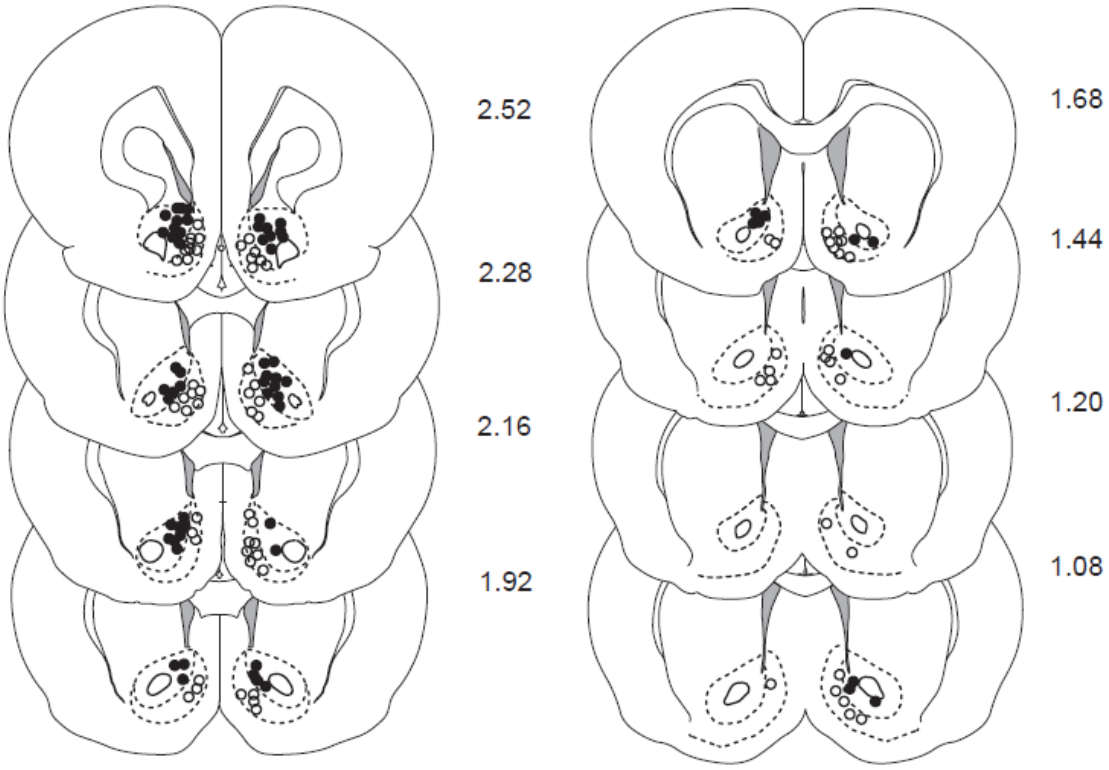

Figure S1. Histological verification of recording array wires in the nucleus accumbens (NAc) core and shell. Marked locations are limited to electrodes that contributed to data presented here. Filled circles indicate electrode locations in the NAc core, and open circles indicate electrode locations in the shell. Numbers to the right indicate anteroposterior coordinates rostral to bregma (mm). A total of 272 wires (16 per animal) were implanted bilaterally and aimed at the NAc core and shell. Of these, we confirmed 98 wires were implanted in the NAc core and 113 wires in the NAc shell. Data from electrodes located outside of the NAc were excluded from analysis. See main text for electrode placement numbers in the core and shell. Images modified from Paxinos and Watson (12) and reprinted with permission from Elsevier.

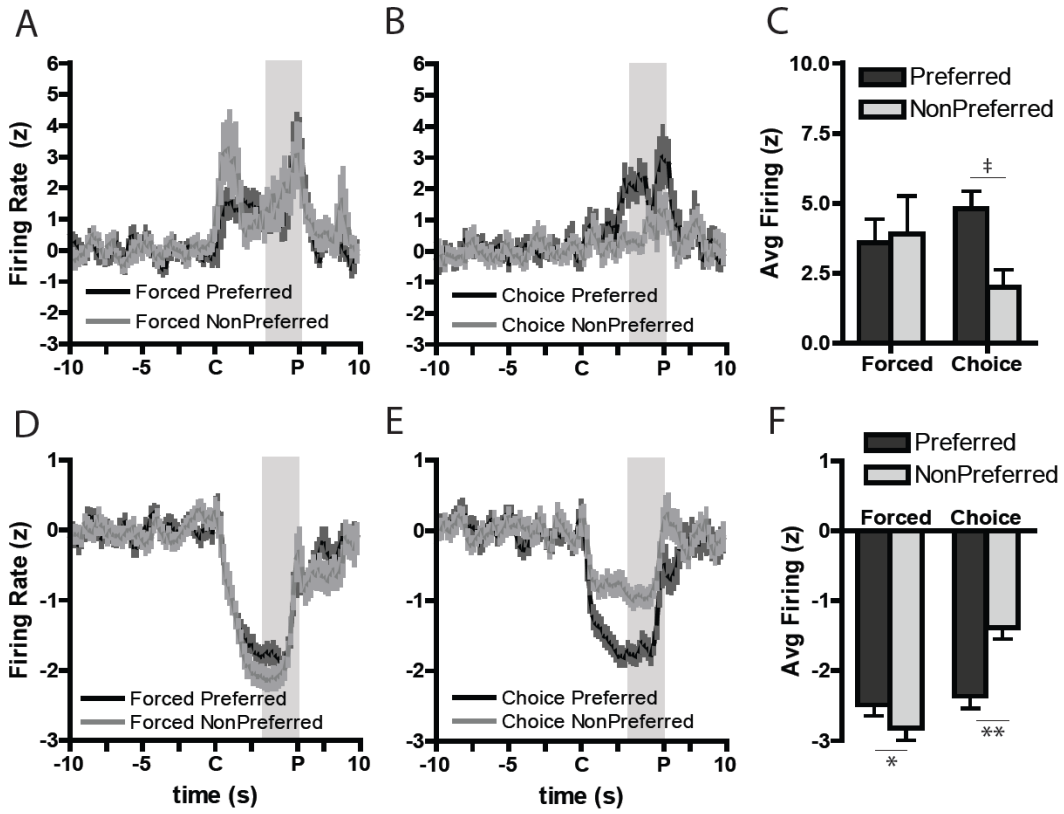

Figure S2. Averaged population responses for excitatory (**A-C**) and inhibitory (**D-F**) prepress phasic neurons in the core. Shaded area indicates 2 s prior to and inclusive of press (P). Tukey, Preferred vs NonPreferred, *$p < 0.05$, **$p < 0.001$; ‡ Wilcoxon, $p = 0.05$.

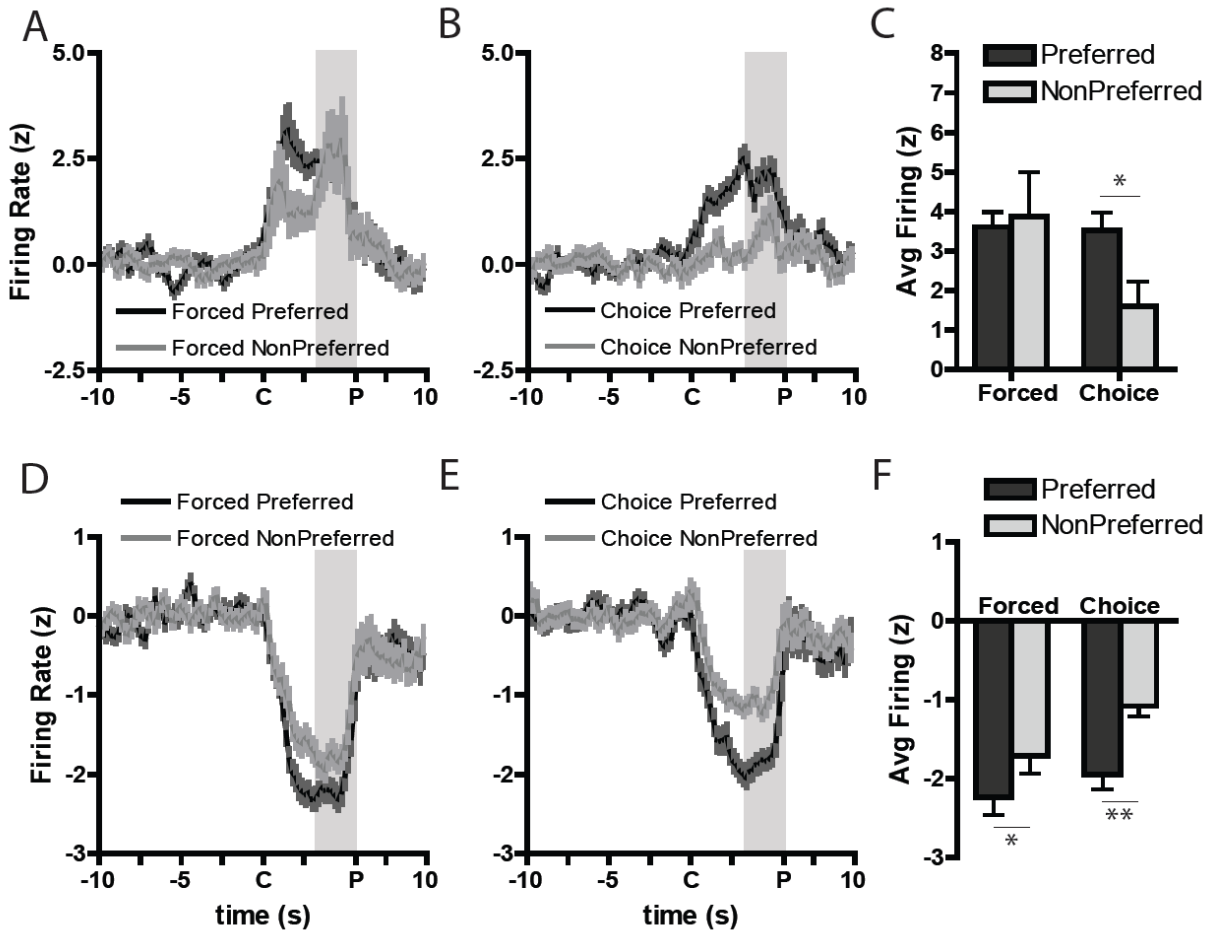

Figure S3. Averaged population responses for excitatory (**A**-**C**) and inhibitory (**D**-**F**) prepress phasic neurons in the shell. Shaded area indicates 2 s prior to and inclusive of press (P). Tukey, Preferred vs NonPreferred, *$p < 0.05$, **$p < 0.001$; ‡ Wilcoxon, $p = 0.05$.

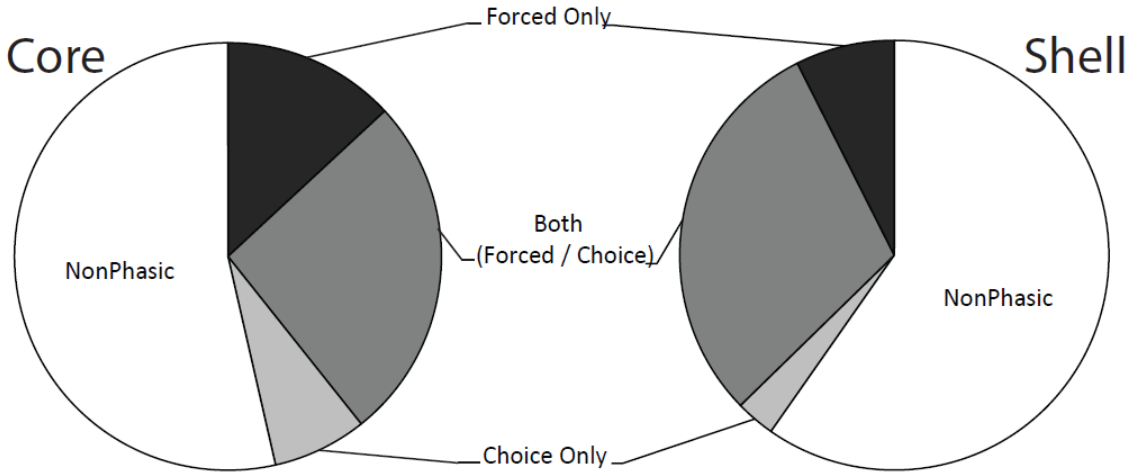

Figure S4. Percent of cells in the core (left) and shell (right) phasic for the preferred prepress periods during forced and choice trials. During the press, there were a large proportion of cells that where phasic during both forced and choice trials. However, there were no differences in the populations of any phasic cell type between core and shell for the different trial types ($p > 0.05$) for all comparisons.

Supplemental References

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