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Progressive and lasting amplification of accumbal nicotine-seeking neural signals

Karine Guillem[1] and **Laura L. Peoples**[2,✉]

[1]Department of Integrative Neurophysiology, CNCR, VU University, Amsterdam, Netherlands
[2]Department of Psychiatry, TRL, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Abstract

Although neuroadaptations in the nucleus accumbens (NAc) are thought to contribute to nicotine addiction, little is known about the chronic effects of nicotine on NAc neuronal activity. In the present experiment, rats were exposed to a 23-day period of nicotine self-administration (SA), a 30-day abstinence period, and a 7-day period of re-exposure to SA. Chronic electrophysiological procedures were used to record the activity of individual NAc neurons on the 3rd and 23rd day of initial SA and on the 1st, 3rd, and 7th day of re-exposure. Between-session comparisons showed that NAc neurons exhibit two patterns of plasticity under the present experimental conditions. First, phasic-increase firing patterns time-locked to the nicotine-reinforced lever-press do not change during initial SA, but then show increases in prevalence and amplitude post-abstinence, which persist during re-exposure. Second, for neurons that show no phasic response time-locked to the nicotine-reinforced lever-press, average baseline and SA firing rates decrease during initial SA, return to normal during abstinence, and decrease again during re-exposure. As a combined consequence of the two types of neurophysiological plasticity, average firing rate of NAc neurons at the time of nicotine-directed behavior undergoes a progressive and persistent net amplification, across the successive stages of SA, abstinence, and re-exposure. This net increase in NAc firing at the time of nicotine-directed behavior occurs with an increase in animals' motivation to seek nicotine. The adaptations which occur in nicotine-exposed animals do not occur in animals exposed to sucrose. The NAc neurophysiological plasticity potentially contributes to compulsive tobacco use.

Keywords

extracellular recording; nicotine addiction; self-administration; abstinence; nucleus accumbens; neuroadaptation

INTRODUCTION

Tobacco is a drug of significant abuse worldwide, with serious associated pathologies and costs to society (Shiffman et al., 1998; Henningfield et al., 2000). Given adequate drug exposure, individuals develop an addiction to nicotine, the primary active ingredient of tobacco. Drug addiction is a progressive and chronic disorder: Individuals gradually transition from controlled to compulsive drug seeking and taking, and exhibit a high incidence of relapse even after extended periods of abstinence (Lerman et al., 2007). It is hypothesized that the development and chronicity of compulsive nicotine-directed behavior are mediated by drug-induced

✉ Corresponding author: Department of Psychiatry, TRL, University of Pennsylvania, Philadelphia, Pennsylvania 19104 Tel: 215-573-6450; Fax: 215-735-4500 lpeoples@mail.med.upenn.edu .

neuroadaptations, which progress and persist across cycles of drug taking and abstinence (Koob and Le Moal, 1997; Nestler and Aghajanian, 1997; Everitt and Wolf, 2002; Nestler, 2004). The NAc is considered a key structure underlying addiction to nicotine, and other drugs (Stein et al., 1998; Koob and Le Moal, 2001; Everitt and Wolf, 2002; Brody et al., 2004). Based on these observations, identifying nicotine-induced NAc neuroadaptations that develop and persist across cycles of drug taking, abstinence and re-exposure is an important step toward understanding and ultimately treating drug addiction.

In the present study, chronic electrophysiological recordings were used to test for nicotine-induced adaptations (i.e., plasticity) in NAc neuronal activity that develop and persist across stages of nicotine SA, abstinence and re-exposure. The recordings showed that NAc neurons undergo unique plasticity during stages of drug taking versus extended abstinence (i.e., referred to as stage-specific plasticity). The neurons also exhibit forms of plasticity that vary depending on the response of the neurons during daily self-administration sessions (i.e., referred to as activity-dependent plasticity). The nicotine-induced stage-specific and activity-dependent plasticity is associated with a progressive and persistent net increase in neuronal activity associated with nicotine-directed behavior, as well as with an enhanced motivation to engage in nicotine-directed behavior. The observed NAc neurophysiological plasticity expands our understanding of nicotine-induced plasticity, particularly with respect to nicotine SA, abstinence and re-exposure. The findings are potentially relevant to identifying mechanisms that contribute to compulsive nicotine-directed behavior.

MATERIALS AND METHODS

Subjects

Subjects were 18 male Long-Evans rats. Protocols were in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. Public Health Service and approved by the Animal Care and Use Committee of the University of Pennsylvania.

Surgery and post-operative maintenance

Animals were deeply anesthetized with ketamine (30 mg/kg IP) and xylazine (5 mg/kg IP) and an indwelling catheter (Silastic tubing: 0.012 inch inner diameter, 0.025 inch outer diameter; Dow Corning Corporation, Midland, Michigan, USA) was surgically implanted into the external jugular vein. The catheter was secured to the vein with surgical silk sutures and passed subcutaneously to the top of the back where it exited into a connector (modified 22 gauge cannula). Under isofluorane anesthesia, arrays of 16 teflon-coated stainless steel microwires were bilaterally implanted in the accumbens [AP: +0.7 to +2.7 mm, ML: ±0.8 to ±2.2 mm, relative to bregma and DV: − 6.8 to −7.2 mm relative to level skull (Paxinos and Watson, 2004)]. A stainless steel ground wire was also implanted 4 mm into the ipsilateral side of the brain, 5 mm caudal to bregma. Animals had free access to water but were restricted to 15-20 grams of food each day to maintain body weight at ~360-380 grams. A detailed description of surgical and post-operative procedures was described in an early report (Peoples, 2003; Guillem et al., 2005). After surgery, animals were flushed daily with 0.2 ml of an ampicillin solution (0.1 g/ml) containing heparin (300 IU/ml) to maintain patency.

Chronic extracellular recordings

Procedures—Voltage signals from each microwire were recorded, amplified up to 32000X, processed, and digitally captured using commercial hardware and software (Plexon, Inc, Dallas, TX). Single units were discriminated off-line with principal component analysis (Offline Sorter, Plexon, Inc, Dallas, TX). The quality of individual-neuron recordings was ensured with the following criteria: <3% of all interspike intervals exhibited by the unit were shorter than 1000 μs, and the average amplitude of the unit waveform was at least three times

larger than that of the noise band. The average amplitude of recorded neural waveforms was 156 μV. Electrophysiological data were analyzed using NeuroExplorer (Plexon) and Matlab (Mathworks, Natick, MA).

Between-group and between-session stability of electrophysiological recordings—Average waveform amplitude and number of neurons recorded per animal were quantified for all recording sessions. Comparisons of these measures between days and groups showed that the characteristics of the electrophysiological recordings were stable across the different recording sessions, and were comparable between the nicotine and sucrose groups (Supplemental Materials, Fig. 1).

Operant-conditioning apparatus

Behavioral procedures were carried out in operant chambers housed inside sound–attenuating cubicles. Chambers were equipped with a retractable lever, a house light mounted on the ceiling, a signal light above the response lever, a white noise-generator, and a tone generator. Operant equipment, hardware, and control software were purchased from Med-Associates, Inc., (St. Albans, VT).

Experimental procedures

Nicotine and sucrose SA sessions—For the nicotine group, the start of each nicotine SA session was signaled by the illumination of a house light and the insertion of a lever. Animals self-administered the nicotine reinforcer on a fixed-ratio 1 (FR1) schedule of reinforcement. Each reinforced lever-press was followed by an intravenous nicotine infusion (i.e., 30 μg/kg per infusion, free base, in 0.2 ml over 7.5 s), a 10-s tone, a 10-s illumination of the light above the lever, and the retraction of the lever. A 60-s timeout preceded the reinsertion of the lever and the start of the next trial. At the end of the session, the house light was extinguished and the lever was retracted. Each SA session was preceded and followed by a 60-min period during which no session events occurred. For the sucrose group, sucrose SA sessions were conducted in an identical manner to the nicotine SA sessions, except for the following: 1) each reinforced lever-press was followed by delivery of sucrose solution into a drinking well (i.e., 0.2 ml of 32% sucrose over 10 s), instead of an intravenous nicotine infusion, and 2) the number of sucrose infusions was matched to the daily number of nicotine infusions earned by the nicotine group.

Progressive-ratio sessions—Behavioral responding was examined in two progressive-ratio self-administration sessions. In these sessions, the FR response requirement for reinforcement was increased according to the following sequence: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, etc. (Richardson and Roberts, 1996). The progressive-ratio sessions lasted for either a maximum of 6 h or until 1 h elapsed without a reinforcer delivery. The last ratio completed during the session was defined as the breaking point.

Training and SA history—After 1 week of recovery from surgery, animals were exposed to three daily habituation sessions. In each session, animals were placed in the chamber for 4 h and connected via a cable to the fluid/electronic swivel. Thereafter, animals were trained to self-administer nicotine (n = 10) under a FR1 schedule of reinforcement in daily 2-hr sessions for 23 days. After Day 23 of the initial SA stage, animals were exposed first to one additional day of FR1 SA training, and then on the next day to one progressive-ratio session (i.e., on day 25 of SA). Then, animals underwent a 1-month abstinence period during which drug access was interrupted and animals remained in their home cages. Following the abstinence period, animals were re-exposed to daily nicotine SA sessions for 7 days. Thereafter, animals were exposed first to one additional FR1 SA training session and then to a progressive-ratio session. The sucrose animals (n = 8) were similarly treated.

Recording sessions

Electrophysiological recordings were conducted during the last habituation day and during the 3rd and 23rd day of the initial SA stage. Additional recordings were conducted on the 1st, 3rd, and 7th day of re-exposure. The experimental events of recording sessions conducted on SA training days were the same as those during all other SA training days.

Analysis of firing rates and firing patterns

Normalization of firing rate data—A preliminary analysis of firing rates showed that they were skewed. Therefore, for all between-group and between-session comparisons, firing rates were normalized using the transformation of $\log_{10}(x+1)$ to reduce the skew of the firing-rate distribution, and thereby allow for parametric ANOVA statistics. All average values are reported as mean ± the standard error of the mean (± SEM).

Phasic increase in firing time-locked to the reinforced lever-press—Each neuron was tested for a short-duration phasic increase in firing time-locked to the reinforced lever-press. These firing patterns were identified using lever-presses that occurred during the maintenance phase of the SA session, when response rates were stable (i.e., last 15 presses). Across these 15 trials, the average firing rate during the 1 s pre-press was compared to the average firing rate during a baseline period (i.e., −12 to −9 s before the lever-press) using a Wilcoxon test. A comparable comparison was made between the 1 s post-press and the −12 to −9 s pre-press. A phasic increase in firing time-locked to the reinforced lever-press was defined as a significant increase in the 1 s pre-press, the 1-s post-press, or both. Preliminary analyses of between-session changes in phasic activity showed that those changes were comparable for the various types of phasic-increase firing patterns (data not shown). The neurons are therefore treated as a single group in all analyses described in RESULTS.

Prevalence and magnitude of phasic firing patterns—For each animal and recording session, we determined the percent of recorded neurons that exhibited a phasic-increase firing pattern time-locked to the reinforced operant response, and averaged those numbers to calculate the mean percent of phasic-increase firing patterns per animal. The magnitude of the firing pattern was calculated using the following ratio: $|A\text{-}B|/(A+B)$. The variable *A* equaled the average firing rate (i.e., Hz per 0.1 s bin) during the 1 s pre-press, the 1 s post-press, or the 1 s pre- and post-press, depending on whether neurons exhibited respectively, an exclusively pre-press, an exclusively post-press, or a combined pre- and post-press firing pattern time-locked to the reinforced press. The variable *B* equaled the average firing rate (i.e., Hz per 0.1 s bin) during the −12 to −9 s pre-press. Changes in prevalence and magnitude of phasic firing patterns were assessed using ANOVA.

Histology and core versus shell comparisons

Histological procedures were used to identify the location of all wire tips used to record neurons. Under anesthesia, anodal current (50 μA for 5 s) was passed through each microwire. Animals were then perfused with 4% paraformaldehyde in 0.9% saline. The brains were cut into 50 μm coronal sections which were mounted on slides and incubated in a solution of 5% potassium ferricyanide and 10% hydrochloric acid to stain the iron deposits left by the recording tips. The tissue was counterstained with 0.2% solution of neutral red. The location of each wire tip was plotted on the coronal plate that most closely corresponded to its anterior-posterior position. Only those neurons which were located within the boundaries of the NAc were included in analyses (Fig. 1).

To test for an effect of NAc sub-territory the location of each neuron was assessed relative to the core – shell boundaries defined in Paxinos and Watson (2004). All neurons identified as core and shell neurons were a minimum of 0.5 mm from the core-shell border. A preliminary

ANOVA analysis showed that the prevalence of core and shell neurons was comparable between the sucrose and nicotine groups and stable across the successive recording sessions (Supplemental Materials, Fig. 2).

RESULTS

Nicotine group

Nicotine intake during daily SA training sessions—Nicotine intake progressively increased during the initial SA stage from 15.1 ± 1.4 infusions to 32.5 ± 1.5 infusions (i.e., day 1 versus day 23), but remained stable thereafter (Fig. 2A). A one-way ANOVA with day (i.e., 3rd and 23rd days of initial SA and 1st, 3rd, and 7th day of re-exposure) as a factor showed a significant effect of day on nicotine intake ($F_{(29,261)} = 8.14$; $p < 0.001$). Post-hoc comparisons showed that the daily rate of nicotine infusion started to increase significantly relative to the 1st SA session, by the 7th session ($p < 0.05$) and continued to increase until the 12th session. Thereafter, daily nicotine intake remained stable (NS; session 12 compared to session 23). Moreover, nicotine intake during re-exposure was not significantly different from that on the last day of initial nicotine SA (NS; sessions 1-7 of re-exposure compared to session 23 of initial nicotine SA).

Incentive to engage in nicotine-directed behavior—Substantial evidence suggests that abstinence from drugs of abuse leads to an increase in drug-directed behaviors (Tran-Nguyen et al., 1998; Grimm et al., 2001; Di Ciano and Everitt, 2002; George et al., 2007; O'ell L and Koob, 2007). We tested for a similar pattern of behavior with between-day comparisons of the following two measures: 1) latency to the first nicotine self-infusion during each of the recorded SA sessions, and 2) breaking-point for nicotine self-administration, which was determined in two progressive-ratio sessions. The first progressive-ratio session was conducted after the 23rd day of initial SA and prior to the onset of the abstinence stage (i.e., on day 25 of SA). The second was conducted after the 7th day of re-exposure (i.e., on the 9th day of re-exposure).

A one-way ANOVA with day (i.e., 3rd and 23rd days of initial SA and 1st, 3rd, and 7th days of re-exposure) as a factor showed that there was a significant between-session decrease in the average latency to the first nicotine self-infusion ($F_{(4,36)} = 3.32$; $p < 0.05$). Post-hoc comparisons showed that the latency to the first infusion was significantly lower during each of the nicotine re-exposure sessions relative to the 3rd and 23rd days of the initial nicotine SA stage (Fig. 2B). Furthermore, a one-way ANOVA showed that the average breaking-point increased between the pre-abstinence progressive-ratio session (i.e., 15.3 ± 2.43 presses) and the post-re-exposure progressive-ratio session (i.e., 45.2 ± 6.99 presses) ($F_{(1,9)} = 19.85$; $p < 0.01$).

Stage-specific and activity-dependent plasticity in NAc neuronal activity in animals exposed to nicotine SA

Stage-specific changes in phasic-increase firing patterns time-locked to the nicotine-reinforced lever-press—Each neuron was tested for a short-duration phasic increase in firing rate time-locked to the reinforced lever-press. On day 3 of initial nicotine SA, 19.5% (17/87) of the NAc neurons exhibited a significant phasic increase in firing rate time-locked to the nicotine-reinforced lever-press (Fig. 3A-C). We compared the percentage and the magnitude of the phasic firing patterns across initial nicotine SA, abstinence, and re-exposure (Fig. 4A-B). One-way ANOVAs showed that there was a significant effect of day on both the percentage ($F_{(4,45)} = 3.23$; $p < 0.05$) and the magnitude of the phasic-increase firing patterns ($F_{(4,82)} = 4.01$; $p < 0.01$). Post-hoc comparisons revealed that there was no significant change in either the incidence or the amplitude of the phasic increase firing patterns time-locked to the reinforced press between the 3rd and the 23rd days of initial nicotine SA. However,

the amplitude and the incidence of the firing patterns were significantly increased (≥140%) during each of the 3 re-exposure recording sessions, relative to the 3rd and 23rd days of initial nicotine SA ($p < 0.05$).

A stage-specific decrease in average baseline and SA firing rates—For all recorded neurons combined, between-session comparisons were made of average firing rate during the 60-min pre-session baseline period (i.e., referred to as baseline firing rate) (Fig. 4C). Between-session comparisons were also made of the average firing rate during the SA session (i.e., referred to as SA firing rate) (Fig. 4D). A two-way ANOVA with recording day (i.e., habituation, 3rd and 23rd days of SA and 1st, 3rd and 7th days of re-exposure) and session phase (i.e., baseline versus SA) as factors showed a significant effect of recording day ($F_{(5,461)} = 6.79$; $p < 0.001$), a significant effect of session phase ($F_{(1,461)} = 22.24$; $p < 0.001$), and a significant interaction between recording day and session phase ($F_{(5,461)} = 2.96$; $p < 0.05$).

A number of post-hoc comparisons were conducted to further characterize changes in the average firing rates of the task-non-activated neurons. Comparisons between baseline and SA firing rates showed that average SA firing rate was significantly lower than average baseline firing rate on several days, including the 3rd ($p < 0.01$) and 23rd ($p < 0.001$) days of initial SA, and the 7th day of re-exposure ($p < 0.01$). Between-session comparisons showed that the average baseline firing rate decreased significantly between the habituation day and the 23rd day of nicotine SA ($p < 0.05$). After 30 days of abstinence (i.e., on day 1 of re-exposure), average baseline firing rate returned to that observed in drug-naïve animals (i.e., did not differ significantly from average baseline firing rate observed during the habituation recording session). Hypoactivity re-emerged during re-exposure: By the 3rd day of re-exposure average baseline firing significantly decreased relative to the 1st day of re-exposure ($p < 0.05$), and equaled the average firing rate on the 23rd day of initial nicotine SA. Changes in average SA firing rate followed the changes in average baseline firing rate (Fig. 4D).

An activity-dependent decrease in average baseline and SA firing rates

Different between-session changes in the average firing rates of two sub-sets of neurons: In a previous cocaine SA study (Peoples et al., 2007), we found that NAc neurons underwent different between-session changes in average baseline and SA firing rates, depending on whether the neurons showed a phasic increase in firing rate time-locked to the cocaine-reinforced lever-press during daily SA sessions. Specifically, neurons that showed a phasic increase in firing time-locked to the cocaine-reinforced lever-press during daily SA sessions, as a group showed no between-session change in either average baseline or SA firing rate across a 30-day period of cocaine SA. However, neurons that did not show a phasic change in firing rate time-locked to the cocaine-reinforced lever-press, as a group showed a significant decrease in average baseline and SA firing rates across the same 30-day period of cocaine self-administration. In the present study, we investigated whether neurons showed a similar 'activity-dependent' effect across the stages of initial SA, abstinence, and re-exposure.

Neurons in the nicotine group were sorted into two categories: 1) neurons that showed a phasic increase in firing time-locked to the nicotine-reinforced lever-press (i.e., referred to as task-activated neurons) (Figs. 3 A-C and 5, top row) and 2) neurons that did not show a phasic change in firing rate time-locked to the nicotine-reinforced lever-press (i.e., referred to as task-non-activated neurons) (Figs. 3D and 5, bottom row). A between-session comparison was made of the average baseline and SA firing rates of these two groups of neurons (Fig. 4E-F). A three-way ANOVA with activation (i.e., task-activated versus task-non-activated), day (i.e., 3rd and 23rd days of SA and 1st, 3rd, and 7th days of re-exposure) and session phase (i.e., baseline versus SA) as factors showed that there was a significant effect of activation ($F_{(1,311)} = 4.34$; $p < 0.05$), as well as a significant interaction among activation, recording day, and session phase

($F_{(4,311)} = 4.12$; $p < 0.05$). These findings show that task-activated and task-non-activated neurons underwent different between-session changes in average firing rates.

Additional analyses were conducted to characterize the different changes in average firing of the task-activated and task-non-activated neurons. A two-way ANOVA applied to the task-activated neurons showed no effect of recording day ($F_{(4,72)} = 0.78$, NS) and no interaction between recording day and session phase ($F_{(4,72)} = 1.28$; NS), indicating that the average firing of the task-activated neurons did not show any between-session change in average baseline or SA firing rate (Fig. 4E, also see Fig. 5, top row). In contrast, a two-way ANOVA applied to the task-non-activated neurons showed a significant effect of day ($F_{(4,239)} = 3.95$; $p < 0.01$), and no significant interaction between recording day and session phase ($F_{(4,239)} = 1.23$, NS) (Fig. 4F), indicating a significant change in both the baseline and SA average firing rates of the task-non-activated neurons (Fig. 4F, also see Fig. 5, bottom row). Additional between-day comparisons showed that the average baseline and SA firing rates of the task-non-activated neurons decreased significantly between the 3rd and the 23rd day of initial SA. Moreover, average firing rates decreased significantly on the 7th day of re-exposure relative to both the 3rd day of initial SA and the 1st day of re-exposure ($p < 0.05$). An additional analysis of firing exhibited by the task-non-activated neurons showed that the between-session decreases in the average firing rates of the task-non-activated neurons were associated with parallel decreases in burst firing (Supplemental Materials, Fig. 3).

The between-session changes in the average firing rates of the task-non-activated neurons were not attributable to between-session changes in behavior: One interpretation of the between-session decrease in the average firing rates of task-non-activated neurons is that repeated drug exposure altered the frequency of a particular behavior, which in turn led to a change in afferent input to the recorded neurons and a corresponding decrease in the average firing rate of the neurons (i.e., the decrease in firing rate potentially reflects behavioral feedback). The baseline and SA phases are behaviorally distinct. For example, animals engage in locomotion frequently during the SA session and rarely during the baseline phase (experimenter observation). Animals also engage in operant behavior during the SA phase but not during the baseline phase. The comparable between-session changes in baseline and SA firing rates, despite the between-phase differences in behavior, is inconsistent with a primary causal role of behavioral feedback in the firing-rate changes. Nevertheless, we conducted an additional analysis to more directly test the interpretation.

In the analysis, the calculation of average SA firing rate during each SA session was limited to periods in which animals engaged in a particular behavior (e.g., locomotion away from the response lever). Calculating firing rate in this way removed the contribution of a between-session change in behavioral feedback to the between-session comparison of average SA firing rate (Rank, 1983). The control analysis replicated the original finding of a significant between-session decrease in the average SA firing rate of the task-non-activated neurons, supporting the interpretation that the between-session decreases in the firing rates of task-non-activated neurons do not simply reflect changes in behavioral feedback to the neurons (Supplemental Materials, Fig. 4).

A persistent and progressive net increase in NAc firing time-locked to nicotine-directed operant behavior—Based on the observed changes in phasic-firing patterns and average firing rates, it appears that NAc neuronal activity associated with nicotine-directed behavior undergo a persistent and progressive *net* increase across initial nicotine SA, abstinence, and re-exposure. That is the ratio between average firing at the time of the reinforced press (referred to as signal) and average firing during other ‘background’ periods increases progressively across the successive stages of initial SA, abstinence, and re-exposure.

A population analysis of average signal:background for all recorded NAc neurons was consistent with this interpretation.

To conduct the population analysis, we first calculated for each neuron, the average firing rate for each of the following: 1) the 60-min pre-session baseline period (i.e., background period), 2) the 1 s pre-press, and 3) the 1 s post-press. For each neuron the signal period was defined as follows: 1) the 1 s pre- and post-press, if firing during the two periods did not differ significantly, 2) the 1 s pre-press, if firing during that period was significantly greater than firing during the 1 s post-press, and 3) the 1 s post-press, if firing during the post-press period was greater than that during the 1 s pre-press. The average firing rates during the background and signal periods were defined as the background and signal firing rates, respectively (Fig. 6A).

A one-way ANOVA with recording day (i.e., 3rd and 23rd days of SA and 1^{st,} 3rd, and 7th days of re-exposure) as a factor showed that there was a significant effect of recording day on the signal:background ratio ($F_{(4,342)} = 4.99$; $p < 0.001$). Post-hoc analyses showed that the signal:background ratio was significantly increased on all days relative to the 3rd day of initial nicotine SA (i.e., SA3 versus SA23, $p < 0.05$; versus R1 $p < 0.01$; versus R3, $p < 0.001$; versus R7, $p < 0.001$). It was also greater on the 7th day of re-exposure relative to the 23rd day of nicotine SA ($p < 0.05$) (Fig. 6B-C). Together, these results demonstrate that at a population level, average firing rate of NAc neurons time-locked to nicotine-reinforced behavior underwent a progressive and persistent net increase relative to firing during the drug-free, pre-session baseline phase. Comparable findings were obtained when the analyses were repeated with the background firing rate was defined as average firing during the −12 to −9 s pre-press (Supplemental Materials, Fig. 5A-B).

No difference in the between-session plasticity exhibited by neurons located in the core versus the shell of the NAc

Phasic firing patterns time-locked to the nicotine-reinforced lever-press: There was a trend for core neurons to exhibit a greater increase in the magnitude of phasic activity across recording days (e.g., re-exposure versus initial SA) as compared to shell neurons (Supplemental Fig. 6). Several two-way ANOVA analyses with day (i.e., 3rd and 23rd days of SA, 1st, 3rd, and 7th days of re-exposure) and sub-territory (core *versus* shell) as factors were used to test for an effect of sub-territory on phasic-increase firing patterns time-locked to the nicotine-reinforced press. These analyses showed that the average magnitude of the phasic-increase firing pattern was greater in the core than in the shell. However, there was no significant effect of sub-territory on prevalence of the phasic-increase firing patterns. Moreover, there was no significant effect of sub-territory on between-session changes in either the prevalence or the magnitude of the phasic firing patterns. Sub-territory also did not significantly affect the prevalence of task-non-activated neurons (Supplemental Materials, Fig. 6).

Average baseline and SA firing rates: A three-way ANOVA analysis with day, session phase, and sub-territory as factors was used to test for an effect of sub-territory on average pre-session baseline and SA session firing rates. For all neurons combined, there was no significant effect of sub-territory on average baseline and session firing rates and no significant effect of sub-territory on the between-session changes in average baseline and SA firing rates (Supplemental Materials, Fig. 7A-B). There was a trend for the decrease in average baseline and SA firing rates of task-non-activated neurons to be greater for shell neurons than for core neurons (Supplemental Fig. 7E-F). However, ANOVA analyses showed that there were no significant effects of sub-territory on the average firing rates of either task-activated (Supplemental Materials, Fig. 7C-D) or task-non-activated neurons.

Sucrose control group

Behavior—Sucrose animals were matched to the nicotine animals for number of reinforcers per day so that the between-session pattern of total reinforcers per day for the sucrose group was necessarily the same as it was for the nicotine group. However, the latency to first press and the breaking-point were determined by the subjects, so it was possible to use those measures to test for between-session changes in the motivation of animals to self-administer sucrose. ANOVA analyses showed that the rats in the sucrose group exhibited no change in the average latency to the first sucrose-reinforced press ($F_{(4,28)} = 1.58$, NS) (Fig. 7), and no change in the average sucrose breaking-point between the pre-abstinence progressive-ratio session (92.1 ± 6.98) and the post-abstinence progressive-ratio session (96.3 ± 5.50) ($F_{(1,7)} = 2.31$, NS).

No changes in either phasic-increase firing patterns time-locked to the sucrose-reinforced lever-press or average baseline and SA firing rates—Separate one-way ANOVAs, which included recording day (i.e., 3rd and 23rd days of initial SA and 1st, 3rd, and 7th day of re-exposure) as a factor, showed that there was no significant effect of day on either the prevalence ($F_{(4,35)} = 0.33$, NS) or the magnitude ($F_{(4,24)} = 0.01$, NS) of phasic-increase firing patterns time-locked to the sucrose-reinforced lever-press (Fig. 8A-B). Moreover, a two-way ANOVA analysis of average baseline and SA firing rates with recording day (i.e., habituation, 3rd and 23rd days of initial SA and 1st, 3rd, and 7th day of re-exposure) and session phase (i.e., baseline versus SA) as factors showed that there was no significant effect of either day ($F_{(5,386)} = 0.15$; NS) or session phase ($F_{(1,386)} = 0.08$; NS). There was also no significant interaction between recording day and session phase ($F_{(5,386)} = 0.44$; NS) (Fig. 8C-D). Additionally, a three-way ANOVA analysis of average baseline and SA firing rates with activation (i.e., task-activated versus task-non-activated), recording day (i.e., 3rd and 23rd days of initial SA and 1st, 3rd, and 7th day of re-exposure) and session phase (i.e., baseline versus SA) as factors showed that there was no significant effect of activation ($F_{(1,207)} = 0.11$, NS), and no significant interaction between day and session phase ($F_{(4,207)} = 0.85$, NS). Thus, in the sucrose group, neither the task-activated nor the task-non-activated neurons showed significant between-session changes in average baseline and SA firing rates (Fig. 8E-F).

A population analysis of signal and background firing rates was conducted for the sucrose group. Signal and background firing rates were determined as described for the nicotine group (Fig. 9A, also see Fig. 9C). When background was defined as firing during the 60-min baseline period there was no significant effect of recording day ($F_{(4,316)} = 0.83$, NS) on the signal:background ratio (Fig. 9B). Similar results were obtained when background firing rate was defined as firing during the −12 to −9 sec pre-press (Fig. 9C, Supplemental Materials Fig. 5C-D).

No difference in the between-session plasticity exhibited by neurons located in the core versus the shell—The sub-territorial analyses applied to the nicotine group were also conducted for the sucrose group. None of the analyses revealed a significant difference between the core and shell (Supplemental Material).

DISCUSSION

Major findings: Stage-specific and activity-dependent nicotine-induced changes in NAc neural activity

The present study shows that NAc neurons exhibit stage-specific and activity-dependent plasticity across periods of initial nicotine SA, extended abstinence, and re-exposure. This plasticity consists of two predominant patterns. First, phasic firing patterns time-locked to the nicotine-reinforced lever-press show no change during initial drug taking but then show increases in prevalence and magnitude during abstinence, which persist during re-exposure.

Second, average baseline and SA firing rates of task-non-activated neurons decrease during an initial period of daily nicotine exposure, return to normal during extended abstinence, and decrease again during re-exposure, whereas average firing rates of task-activated neurons remain stable. The decreases in the average firing rates of task-non-activated neurons and the increase in prevalence and amplitude of phasic-increase firing patterns are exhibited by both shell and core neurons. At a population level, the net effect of the two patterns of plasticity is to progressively and persistently increase the net strength of average firing of NAc neurons associated with nicotine-directed behavior. These changes in neural activity are associated with an increase in the motivation of animals to engage in nicotine-directed behavior. The behavioral and neural changes observed in the nicotine group do not generalize to animals exposed to sucrose SA, abstinence, and re-exposure.

Interpretations of the present findings and comparisons to other investigations

The effects of nicotine SA and abstinence on phasic firing patterns and the average firing rates of NAc neurons are strikingly similar to previously reported effects of cocaine SA and abstinence on phasic and average firing (Peoples et al., 1999; Hollander and Carelli, 2005; Peoples et al., 2007). The effects of cocaine re-exposure have not yet been characterized. Nicotine and cocaine have different sites and mechanisms of action (Koob and Le Moal, 2001; Nestler, 2005; Ikemoto et al., 2006). However, both drugs share with other addictive drugs certain acute and chronic neurochemical and molecular NAc effects (Di Chiara and Imperato, 1988; Balfour, 2002; McClung et al., 2004). It is possible that the changes in firing associated with nicotine and cocaine SA and abstinence will generalize to other drugs.

The sucrose reward concentration used in the present study (i.e., 32%) is the maximally reinforcing concentration in rats (Reilly, 1999). Additionally, the sucrose reward was more reinforcing than nicotine in the present study, as indicated by the latency-to-first-press and breaking-point measures. Based on these observations, one might hypothesize that the stable neuronal activity in the sucrose group reflects the high reinforcing efficacy of the sucrose reward and a potentially related ‘ceiling effect’ on NAc neurophysiological plasticity. However, a number of observations argue against this interpretation. First, the prevalence and magnitude of the phasic firing patterns and the average baseline firing rates of the nicotine and sucrose groups were quite similar on the first recording day, suggesting that there was a potential for the phasic firing patterns and average firing rates of the two groups to undergo similar plasticity. Second, a previous study which used a 10% sucrose solution as a reinforcer also failed to observe changes in behavior and NAc neural activity. Third, cocaine is considered more reinforcing than nicotine, and yet exposure to cocaine and nicotine SA are associated with comparable changes in phasic firing patterns and average firing rates. These observations argue against the reinforcer-efficacy and ceiling-effect interpretations. The similar changes in NAc phasic firing patterns and average firing rates in animals exposed to cocaine and nicotine [i.e., present study, Hollander and Carelli, 2005, 2007; Peoples et al., 2007a, b], combined with the absence of those findings in sucrose-exposed animals (i.e., present study; Jones et al., 2008) supports the interpretation that the plasticity in the drug-exposed animals is due to chronic pharmacological effects.

Investigators have hypothesized that certain patterns of exposure to highly palatable foods induce neuroadaptations, which are similar to those induced by addictive drugs. Moreover, the shared neuronal plasticity contributes to compulsive intake of both drug and food rewards (Rada et al., 2005; Volkow et al., 2008). The similar neurophysiological plasticity in the NAc following nicotine and cocaine SA and the absence of that plasticity in sucrose SA [i.e., present study, (Hollander and Carelli, 2005, 2007; Peoples et al., 2007; Jones et al., 2008)] is suggestive of an important difference in the NAc neural plasticity induced by repeated exposure to

addictive drugs versus sucrose. Additional studies are required to fully investigate this possibility.

In previous cocaine studies, the abstinence-induced increase in phasic activity was significantly greater in the core than in the shell. Conversely, the decrease in average firing of task-non-activated neurons was significantly greater in the shell than in the core (Hollander and Carelli, 2007; Peoples et al., 2007). Though there were similar trends in the present nicotine study, there were no significant interactions between sub-territory and effect of initial SA, abstinence, and re-exposure on phasic and average firing. The different findings between the cocaine and nicotine studies could reflect an effect of drug, or an effect of other experimental and analytical differences. However, it is noteworthy that neurons in the present study were more concentrated in the anterior NAc relative to neurons recorded in the cocaine studies. It is possible that the between-study differences in core-versus shell effects corresponds to differences in medial-lateral heterogeneity along the anterior-posterior axis of the NAc (Zahm and Brog, 1992).

Relationship between increases in signal:background and the strength of drug-directed behavior

Animal and human studies show that the NAc contributes to directing and invigorating goal-directed behavior, including drug-directed behavior (Kelley and Stinus, 1985; Blackburn et al., 1987; Pfaus and Phillips, 1991; Salamone et al., 1994; Robbins and Everitt, 2007). Drug-induced adaptations within the NAc are hypothesized to contribute to compulsive drug-directed behavior, which characterizes drug addiction. The contribution of the NAc to the occurrence or strength of a particular behavior relative to other behaviors is hypothesized to reflect the relative activation of particular ensembles of neurons associated with the behaviors (Pennartz et al., 1994). Based on this current understanding of NAc contributions to goal-directed behavior the two types of NAc neurophysiological plasticity observed in the present study and the net increase in firing time-locked to the nicotine-reinforced press (i.e., the increase in signal:background ratio for the nicotine-reinforced press) might be expected to increase the propensity of animals to engage in nicotine-directed behavior.

In the present experiment, nicotine intake did not escalate; however, under continuous limited-access conditions, nicotine SA generally does not show increments post-acquisition, perhaps due to aversive effects of high levels of nicotine (Paterson and Markou, 2004; Kenny and Markou, 2006; George et al., 2007). Nevertheless, the latency-to-first-press and breaking-point measures showed evidence of increased motivation to seek nicotine during the re-exposure phase relative to pre-abstinence nicotine SA sessions. Additionally, previous cocaine studies showed that the decrease in average firing of task-non-activated neurons and the post-abstinence increase in phasic activity are both associated with a concomitant strengthening of cocaine-directed behavior (Hollander and Carelli, 2005, , 2007; Peoples et al., 2007). Based on these observations further investigation of the relationship between drug-directed behavior and NAc signal:background for drug-directed behavior is warranted.

Plasticity in NAc neural activity: Possible mediating mechanisms

The mechanisms underlying the neurophysiological plasticity observed in the present study cannot be specified as of yet. However, repeated nicotine exposure induces a number of changes in neurotransmission, which could contribute to the adaptations in NAc neural activity. For example, nicotine exposure leads to lasting changes in glutamatergic neurotransmission (Mansvelder and McGehee, 2000; Mansvelder et al., 2002) and glutamatergic compounds have been shown to influence both nicotine reinforcement and reinstatement to nicotine-directed behavior (Kenny et al., 2003; Kenny and Markou, 2004; Bespalov et al., 2005; Liechti et al., 2007).

Acute effects of nicotine are also potentially relevant in understanding the NAc neurophysiological plasticity observed in the present experiment. For example, acute activation of nicotine receptors can influence intracellular CA^{++} stores in an activity-dependent manner (Dajas-Bailador and Wonnacott, 2004), and contribute to long-term potentiation (Mansvelder and McGehee, 2000; Saal et al., 2003) and long-term depression (Partridge et al., 2002). Nicotine causes several types of acute changes in neurotransmission, which could either induce or modulate NAc neuroadaptations. For example, in the NAc, acute nicotine increases the release of dopamine (Benwell and Balfour, 1992; Corrigall et al., 1992; Pontieri et al., 1996; Di Chiara, 2000; Lecca et al., 2006), which modulates long-term-depression and long-term potentiation (Kauer and Malenka, 2007; Schotanus and Chergui, 2008). Acute nicotine effects could also contribute indirectly to activity-dependent plasticity by altering the firing rate of neurons (Pennartz et al., 1994; Peoples et al., 2004; Peoples et al., 2007).

Conclusions

The present study provides new insight into NAc neurophysiological plasticity induced by nicotine, particularly in relation to a history of nicotine SA, abstinence and re-exposure. Further investigation of the NAc neurophysiological plasticity observed in the present study might elucidate mechanisms that contribute to the development of compulsive drug-directed behavior toward nicotine and other addictive drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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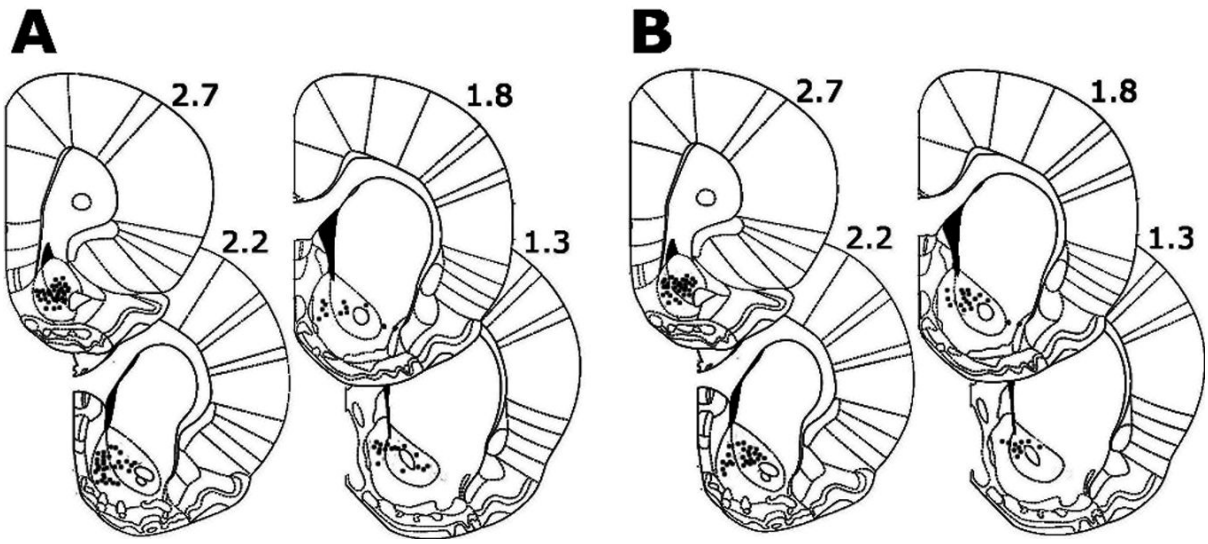

Figure 1. Locations of all recorded neurons in the nicotine and sucrose groups

Locations of all recorded NAc neurons are presented on coronal sections for the nicotine (**A**) and the sucrose (**B**) groups. Numbers indicate millimeters anterior to bregma.

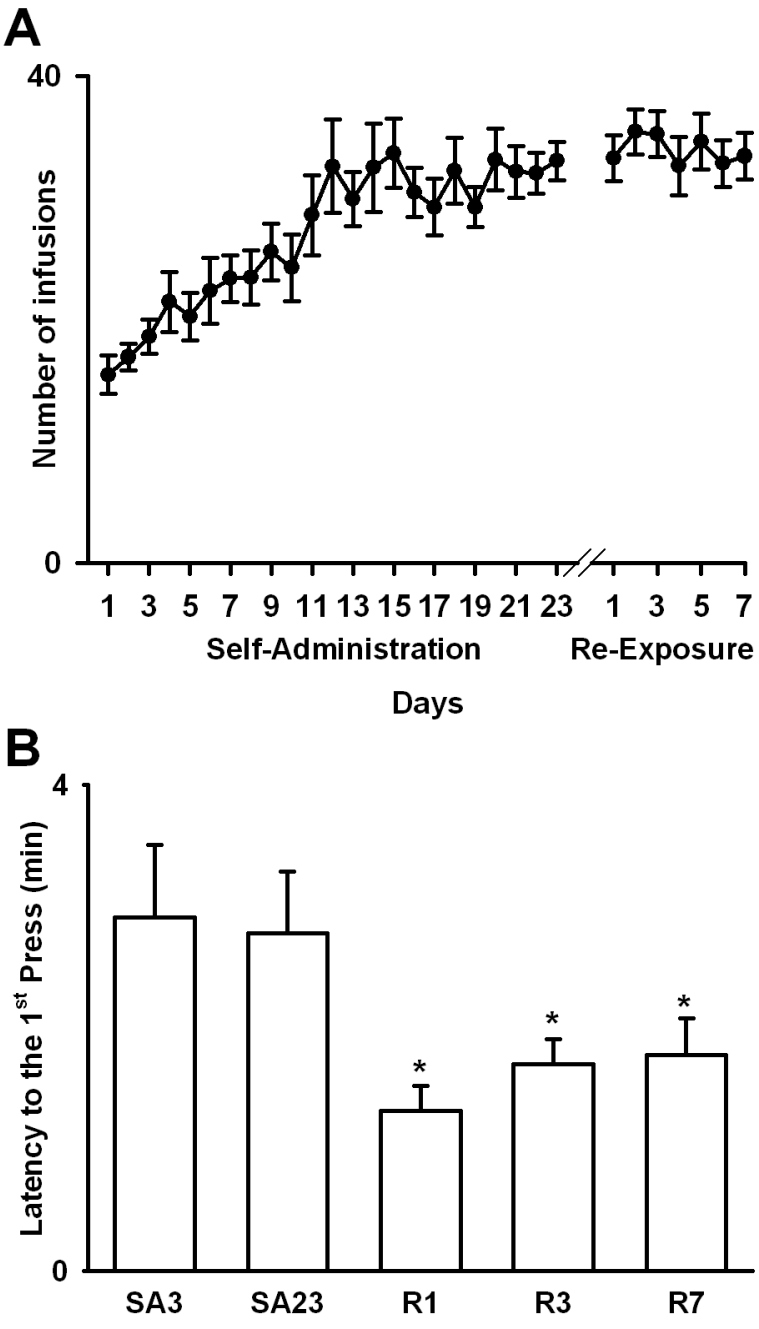

Figure 2. Subjects in the nicotine group showed stable rates of nicotine intake but increased motivation to self-administer nicotine post-abstinence relative to pre-abstinence

A: Average nicotine intake (i.e., total number of infusions per session) during initial nicotine SA and re-exposure is plotted as a function of SA training day (i.e., days 1-23 of initial SA and days 1-7 of re-exposure respectively). **B**: Average latency to first press is plotted as a function of recording day (i.e., Hab = habituation; SA3, SA23 = day 3 and 23 of initial SA stage; R1, R3, and R7 = days 1, 3, and 7 of re-exposure). The symbol * denotes a significant difference ($p < 0.05$) relative to the 3rd and the 23rd days of initial SA.

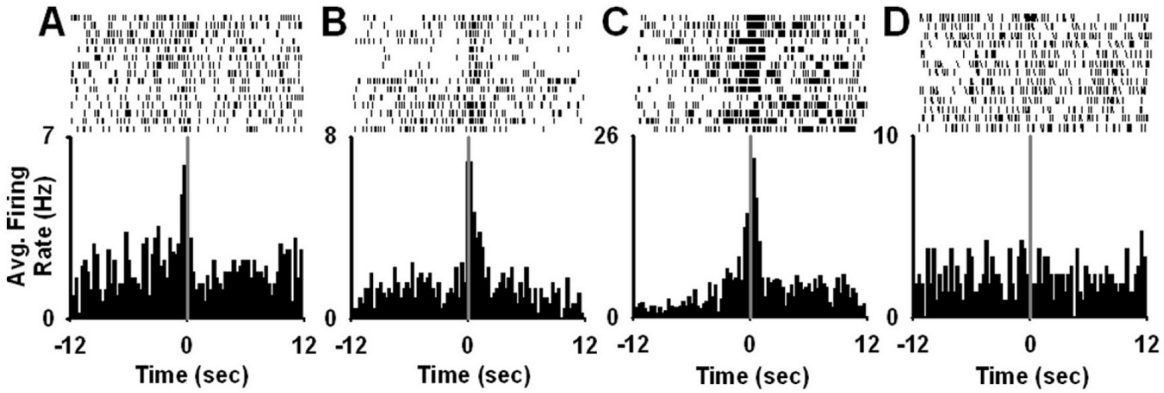

Figure 3. Examples of individual neuron firing patterns time-locked to the nicotine-reinforced lever-press

A-C: Each peri-event histogram shows the average firing rate (i.e., average Hz per 0.1 s bin) of a single neuron plotted as a function of time (s) before and after the nicotine-reinforced lever-press (i.e., 12 s pre-press and 12 s post press). From left to right are shown a pre-press firing pattern (**A**), a post-press firing pattern (**B**) and a combined pre- and post-press firing pattern (**C**); **D:** Average firing rate (i.e., average Hz per 0.1 s bin) of a single task-non-activated neuron during the 12 s before and the 12 s after the nicotine-reinforced lever-press. Above each histogram is shown a trial × trial raster display of firing plotted during the 12 s before and after the nicotine-reinforced lever-press. Individual ticks represent a single discharge of the neuron. Time 0 on the abscissa corresponds to completion of the reinforced lever-press.

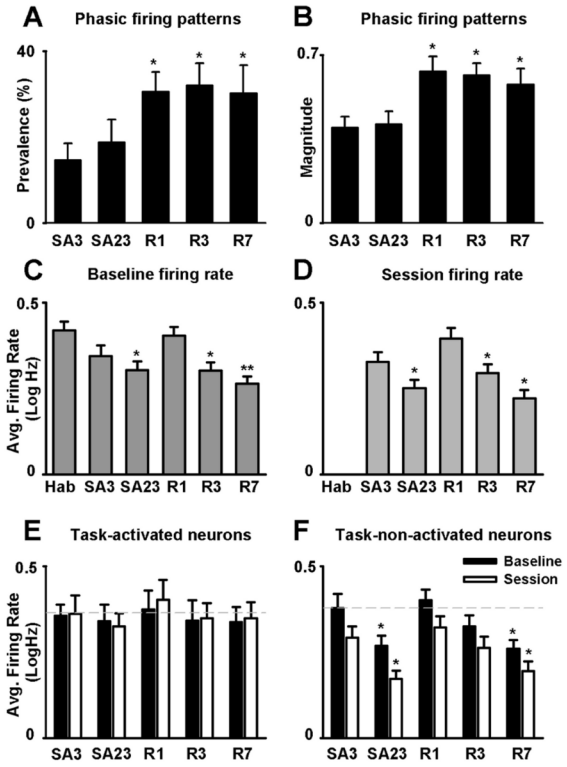

Figure 4. Stage-dependent and activity-dependent changes in NAc firing across the initial nicotine SA, abstinence, and re-exposure stages

A: Average percent of recorded neurons (i.e., per animal), which exhibited a phasic-increase firing pattern time-locked to the nicotine-reinforced lever-press (i.e., prevalence %) is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA session; and R1, R3, and R7 of re-exposure). **B:** Average magnitude of the phasic-increase firing pattern time-locked to the nicotine-reinforced lever-press is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). Calculation of magnitude described in **METHODS**. In both panels A and B the symbol * denotes a significant difference, relative to the 3rd and the 23rd days of SA ($p < 0.05$). **C-D:** Average baseline (**C**) and SA (**D**) firing rates [i.e., average $\log_{10}(x+1)$, abbreviated log Hz] are plotted as a function of recording day (i.e., Hab, SA3, and SA23 of initial SA; and R1, R3, and R7 of re-exposure). The symbols * and ** denote a significant difference relative to habituation day with $p < 0.05$ and $p < 0.01$, respectively. **E-F:** Average baseline and SA firing rates [i.e., average $\log_{10}(x+1)$, abbreviated as log Hz] of task-activated (**E**) and task-non-activated neurons (**F**) are plotted as a function of day (i.e., SA3 and SA23 of initial SA; R1, R3, and R7 of re-exposure). The symbol * denotes a significant difference relative to SA3 ($p < 0.05$).

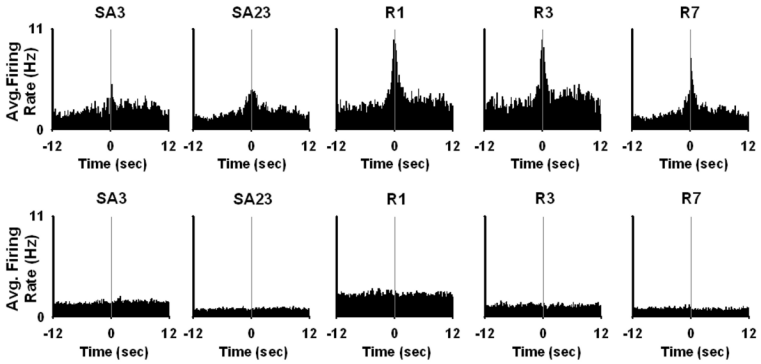

Figure 5. Average firing patterns time-locked to the nicotine-reinforced lever-press: task-activated versus task-non-activated neurons

Top row: The average firing pattern of task-activated neurons time-locked to the nicotine-reinforced lever-press is shown for each SA recording day (i.e., SA3 and SA23 of initial SA; R1, R3, and R7 of re-exposure). In each peri-event histogram, average firing rate (i.e., Hz per 0.1 s bin) is plotted as a function of time (s) before and after the nicotine-reinforced lever-press (i.e., 12 s pre- and 12 s post-press). Time 0 = completion of the operant. **Bottom row:** The average firing pattern of all task-non-activated neurons time-locked to the nicotine-reinforced lever-press is shown for each SA recording day (i.e., SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). In each peri-event histogram, average firing rate (i.e., Hz per 0.1 s bin) is plotted as a function of time (s) before and after the nicotine-reinforced lever-press (i.e., the 12 s pre- and post-press).

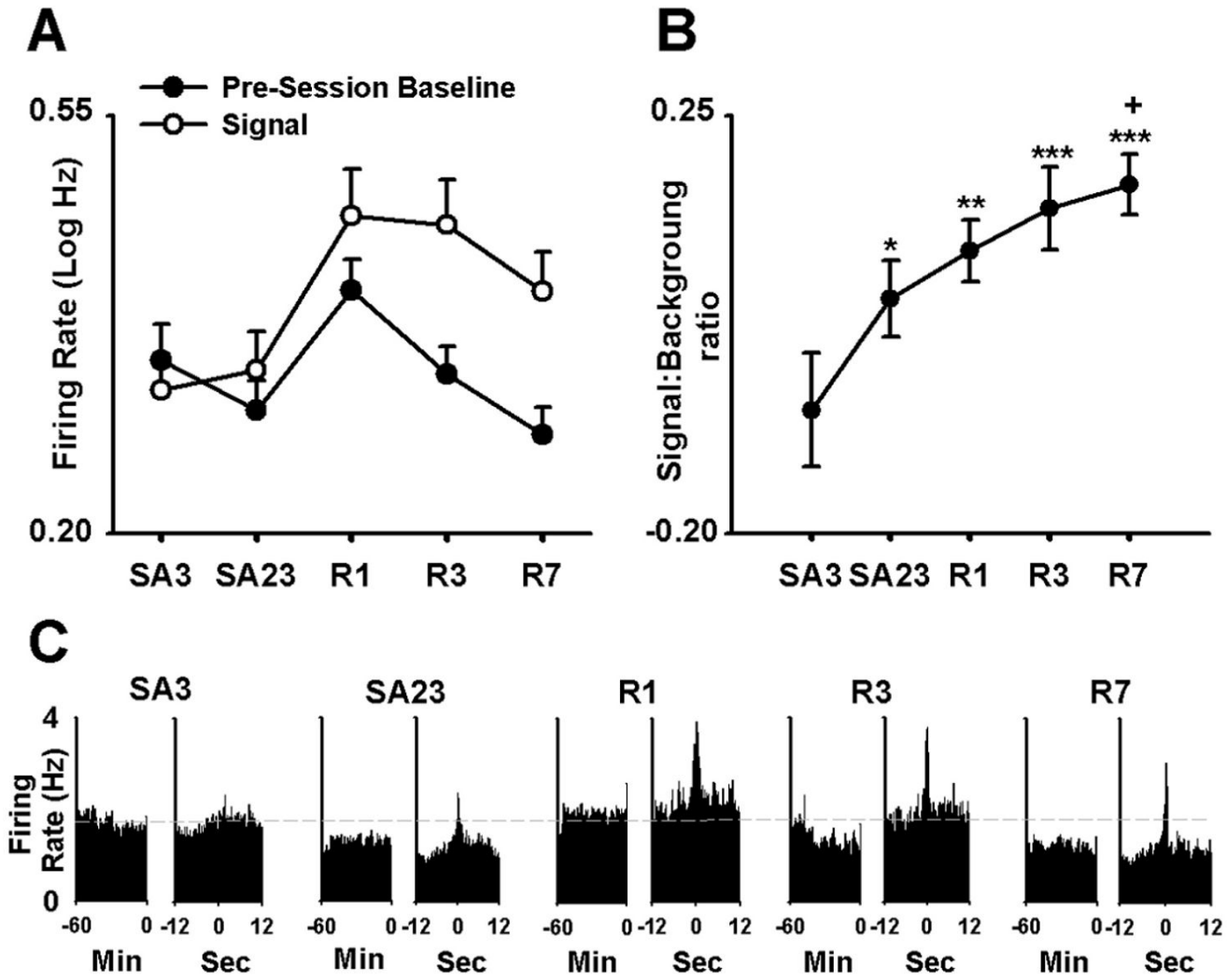

Figure 6. A persistent and progressive net increase in NAc firing time-locked to the nicotine-reinforced lever-press

A: Average signal and background firing rates [i.e., average $\log_{10}(x+1)$, abbreviated log Hz] of all recorded neurons (i.e., all task-activated and all task-non-activated neurons combined) in the nicotine group are plotted as a function of recording day (i.e., SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). The signal firing rate is the average peak firing rate (i.e., signal) time-locked to the nicotine-reinforced press (see **RESULTS**). The background firing rate is average firing rate during the 60-min baseline period. **B:** The average signal:background ratio is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA; R1, R3, and R7 of re-exposure). The symbols *, **, and *** denote a significant difference relative to the 3rd day of initial SA with $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. The symbol + denotes a significant difference relative to the 23rd day of initial SA ($p < 0.05$). **C:** Average baseline and SA firing rates (i.e., average Hz per 0.1 sec bin for all recorded neurons combined) are plotted as a function of recording day (i.e., SA3, and SA23 of initial SA, and R1, R3, and R7 of re-exposure). For each recording day, two average peri-event histograms are shown, one displays average firing rate (i.e., average Hz per 0.1 s bin) during the 60-min baseline period (left histogram of the pair) and the other displays average firing rate (i.e.,

average Hz per 0.1 s bin) during the 12 s before and after the nicotine-reinforced lever-press (right histogram of the pair).

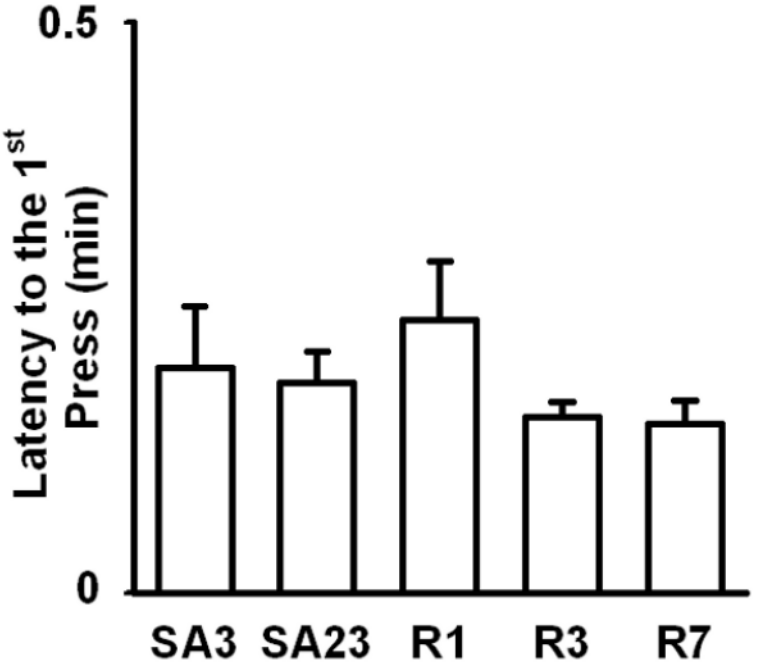

Figure 7. No significant between-session change in latency to the first sucrose-reinforced lever-press

Average latency to the first sucrose-reinforced lever-press is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA, and R1, R3, and R7 of re-exposure).

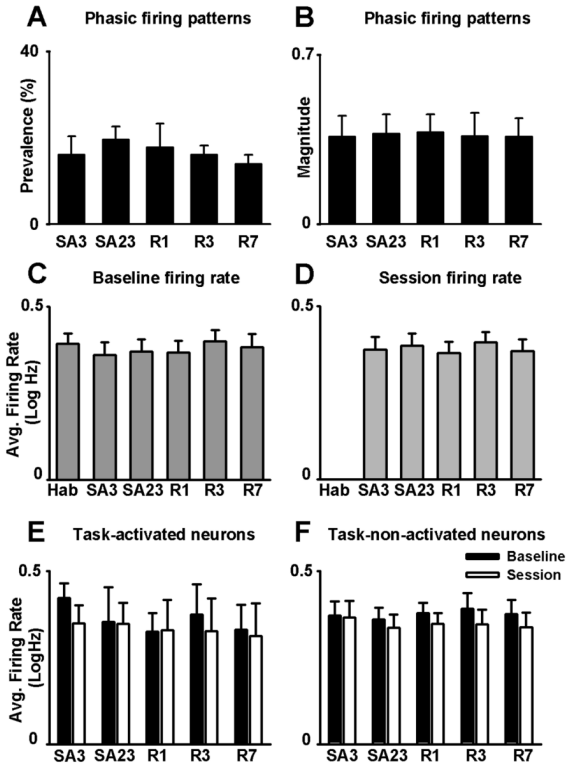

Figure 8. No significant between-session changes in phasic firing patterns and average firing rates in the sucrose group

A: Average percent of neurons (i.e., per animal) exhibiting a phasic-increase firing pattern time-locked to the sucrose-reinforced lever-press (i.e., prevalence %) is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). **B:** Average magnitude of the phasic-increase firing pattern time-locked to the sucrose-reinforced lever-press is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA, R1, R3, and R7 of re-exposure). Calculation of magnitude described in **METHODS**. **C-D:** Average baseline (**C**) and SA (**D**) firing rates [i.e., average $\log_{10}(x+1)$, abbreviated log Hz] are plotted as a function of recording day (i.e., Hab; SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). **E-F:** Average baseline and SA firing rates [i.e., average $\log_{10}(x+1)$, abbreviated log Hz] of task-activated (**E**) and task-non-activated neurons (**F**) are plotted as a function of day (i.e., SA3 and SA23 of initial SA, R1, R3, and R7 of re-exposure).

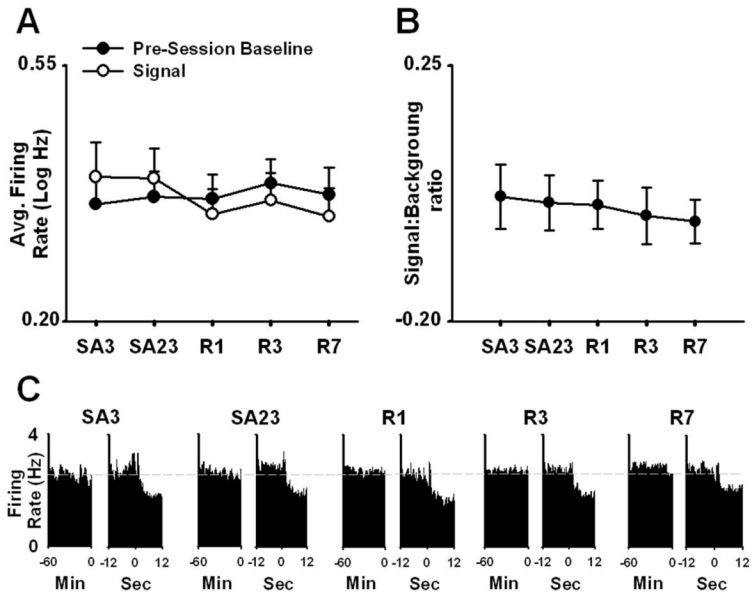

Figure 9. No significant net increase in firing time-locked to the sucrose-reinforced lever-press

A: Average signal and background (i.e., pre-session baseline) firing rates [i.e., average $\log_{10}(x+1)$] of all recorded neurons are plotted as a function of recording day (i.e., SA3 and SA23 of initial SA; and R1, R3, and R7 of re-exposure). **B:** Average signal:background ratio is plotted as a function of recording day (i.e., SA3 and SA23 of initial SA, R1, R3, and R7 of re-exposure). **C:** Average baseline and SA firing rates (i.e., Hz per 0.1 s bin) of all recorded neurons are plotted as a function of recording day (i.e., SA3 and SA23 of initial SA, R1, R3, and R7 of re-exposure). For each recording day two average peri-event histograms are shown, one displays average firing rate (i.e., average Hz per 0.1 s bin) during the 60-min pre-session baseline period (left histogram of the pair) and the other displays average firing rate (i.e., Hz per 0.1 s bin) during the 12 s before and after the sucrose-reinforced lever-press.