

Short communication

Operant behavior during sessions of intravenous cocaine infusion is necessary and sufficient for phasic firing of single nucleus accumbens neurons

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Abstract

The activity of individual accumbens neurons in rats was recorded in relation to intravenous cocaine infusions that were either response (i.e., lever press) contingent or response non-contingent. Neural firing was additionally recorded in relation to non-reinforced lever presses. Comparisons of firing under the three conditions showed that operant behavior was necessary and sufficient for preinfusion firing to occur. Surprisingly, the same was true, in many cases, for firing that occurred during the infusion. For other neurons, firing during the infusion was unrelated to operant behavior and possibly related to infusion stimuli. The relationship to operant behavior exhibited by the majority of NAcc neurons is consistent with previous studies that demonstrated a necessary relationship between NAcc neurons and cocaine reinforced operant behavior.

Keywords: Nucleus accumbens; Psychomotor stimulant; Cocaine; Single neuron recording; Self-administration; Appetitive behavior; Incentive stimulus; Reward

Neurons of the nucleus accumbens (NAcc) make a necessary contribution to the control of cocaine self-administration exhibited by rats [17]. Using chronic extracellular recording techniques, several laboratories have observed changes in firing rate during the few seconds before and after cocaine self-infusion [4–6,10,13]. These phasic firing patterns are potentially related to operant behavior and/or associated stimuli. Alternatively, the firing patterns may be related to other events coincident to the cocaine infusion. The present investigation set up a differentiation between the two alternatives. Specifically, firing patterns associated with response contingent cocaine infusions were compared to firing patterns associated with non-contingent cocaine infusions. A firing pattern related specifically to operant behavior would be expected to be present as long as *and* only if the behavior were present (i.e., present in conjunction with response contingent infusions but not in conjunction with response non-contingent infusions). Phasic firing patterns that are unrelated to the operant and related instead to the infusion (or to some other non-oper-

ant event) would be expected to be present regardless of the presence of the operant behavior.

Twenty-six male Long–Evans rats (maintained at 350 g; Charles River, Wilmington, MA) were chronically implanted with a catheter in the jugular vein and an array of microwires in the NAcc. The surgical and post-operative procedures were described previously [10].

Cocaine self-administration session. Onset of the self-administration session was signaled by illumination of a stimulus light above a response lever. Each lever press activated a pump that infused cocaine (0.69–0.73 mg/kg/0.2 ml) intravenously to the rat. The lever press additionally activated both a 7.5 s tone, which coincided with operation of the pump, and a 40 s time-out, during which the stimulus light was turned off. Rats received an average ($\pm$ standard error) of 21.3 ± 1.5 consecutive days of training (maximum of 60 infusions per day) prior to the electrophysiological recording session.

Response contingent versus non-contingent cocaine infusions. At the beginning of the recording session, subjects self-administered cocaine infusions until response rates stabilized (i.e. loading phase). Thereafter, the session consisted of alternate phases in which infusions were either response contingent or response non-contingent. During

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each contingent phase, infusions (5 or 15) occurred only when the rat depressed the lever. In each non-contingent phase, infusions were activated by the computer, according to the schedule of infusions (± 0.1 ms) self-administered by the rat during the preceding contingent phase. Presented with the non-contingent infusions were the same tone and light stimulus events that had been paired with contingent infusions. Lever presses during the non-contingent phase were non-reinforced and were not paired with the tone and light stimulus events. The total number of non-contingent infusions per session equaled 30. All sessions were videotaped [10].

Electrophysiology and histology. Neural activity was recorded using electrophysiological procedures described previously [10]. Each neuron exhibited a minimum interspike interval consistent with the refractory period of a single neuron and was verified histologically to have been located in the NAcc [10]. Only one recording was obtained from each microwire. Firing patterns were characterized during the 15 s before and after the onset of the cocaine infusion (loading phase excluded). For each neuron, firing that occurred during all response contingent phases was combined into a single histogram that displayed firing in relation to contingent infusions. Data from non-contingent phases were similarly treated. Firing patterns have been verified statistically using the Wilcoxon Matched Pairs test [10].

Behavior. Rats exhibited regular rates of lever pressing during all phases of contingent infusions; self-administration behavior was thus not disrupted by the intervening phases of *non-contingent* infusions (Fig. 1). The regular rates of self-infusion during the contingent phases, combined with the equality of the contingent and non-contingent infusion schedules, maintained calculated drug levels within stable limits throughout the session, assuming constant cocaine kinetics (Fig. 1). Video analysis showed that non-appetitive behavior was the same in both contingent and non-contingent phases and consisted almost exclusively of focused stereotypy. In contrast, the patterns of operant behavior were not the same during the two phases. Rats necessarily pressed the lever during each contingent infusion trial; however, rats pressed the lever, on average, during only 5.4 ± 0.6 of the 30 non-contingent infusion trials (Fig. 1B). On those non-contingent trials in which rats did press the lever, they typically did so within the last 1 min before the scheduled infusion. Trials in which a lever press occurred were excluded from histograms that displayed firing in relation to non-contingent infusions. Correspondingly, the matching trials of the preceding contingent phase were excluded from the histograms that displayed firing in relation to contingent infusions.

Neural sample. During the recording session, 70 single neurons were recorded from 61 microwires in 26 rats. Of the 70 neurons, 29 showed phasic firing time-locked to the reinforced lever press (i.e., were responsive). During the contingent phases, types of phasic firing could be discrimi-

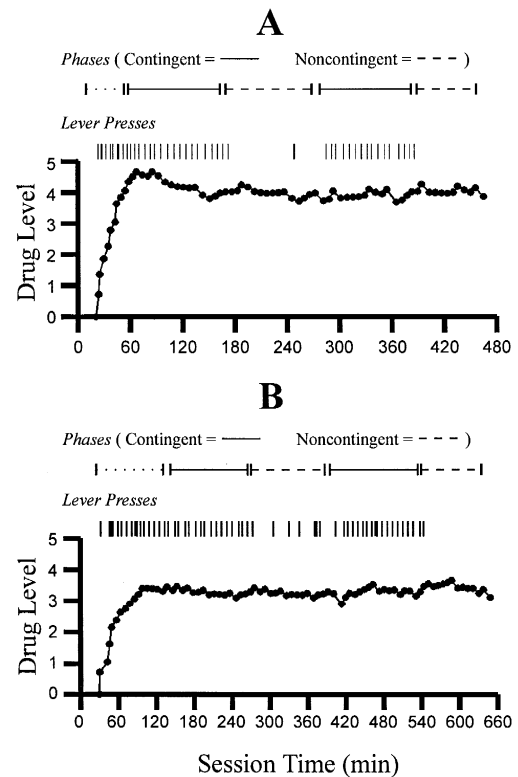


Fig. 1. Pattern of lever presses and calculated drug levels during the alternating phases of response contingent and non-contingent infusions is shown for each of two animals. Within each panel (A and B) is shown the following: each point on the graph shows the calculated drug level (mg/kg) at the time of each single infusion. Drug level is plotted as a function of successive infusions for the initial self-administration (loading) phase, indicated by dotted line, and for the subsequent alternating phases of contingent and non-contingent infusions (the pattern of infusions during the loading phase was not used to determine infusion patterns for non-contingent phases). Consistent with the pharmacokinetics of cocaine in the rat [9], the drug level was calculated assuming first-order pharmacokinetics using the following equation $(B + D)e^{(-kt)}$ [15] in which B = drug level at time of previous infusion; D = infusion dose (mg/kg/inf); t = minutes elapsed between the infusion for which drug level is being calculated and the preceding infusion; k = rate constant for cocaine, derived from half-life (i.e., $k = 0.693/t_{1/2}$) [7] of cocaine in the NAcc of rats administered a single intravenous injection of cocaine [8].

nated based on the timing of firing in relation to the lever press. Analysis of the effect of non-contingent infusions was conducted separately for each type.

Changes in firing rate that began before the operant was completed. During the contingent phase, the largest group of responsive neurons (52%, 15/29) showed an increase in firing that began within -3 s before the operant and continued until 1–3 s after it (but for some neurons as late as 10 s post press) (Fig. 2A_a and B_d). The increase exhibited by most of the neurons (13/15) was sub-typed according to whether the increase occurred *predominantly pre press* ($n = 1$); *predominantly post press* ($n = 6$), or was *symmetrical* relative to the press ($n = 6$). Eight other neurons showed *decreases* in firing (i.e., 3/8 were predominantly pre press, 1/8 were predominantly post press, and 3/8 were symmetrical) (Fig. 2C_g and D_j).

The increases and decreases in firing were affected similarly by the non-contingent phases. On non-contingent infusion trials, all predominantly pre-press patterns (4/4 neurons), almost all symmetrical patterns (8/9 neurons), and about half of the predominantly post-press patterns (3/7 neurons) were *completely absent when the operant was absent* (Fig. 2A_a vs. Fig. 2A_b; Fig. 2C_g vs. Fig. 2C_h).

For the remaining neurons (1/9 symmetrical neurons and 4/7 predominantly post-press neurons), the pre-press firing was absent but the post-press firing either did not change or was only diminished (Fig. 2B_d vs. Fig. 2B_e; Fig. 2D_j vs. Fig. 2D_k). These data show that all pre-press firing and, in most cases, post-press firing that accompanied it were related to the occurrence of the operant. Consistent

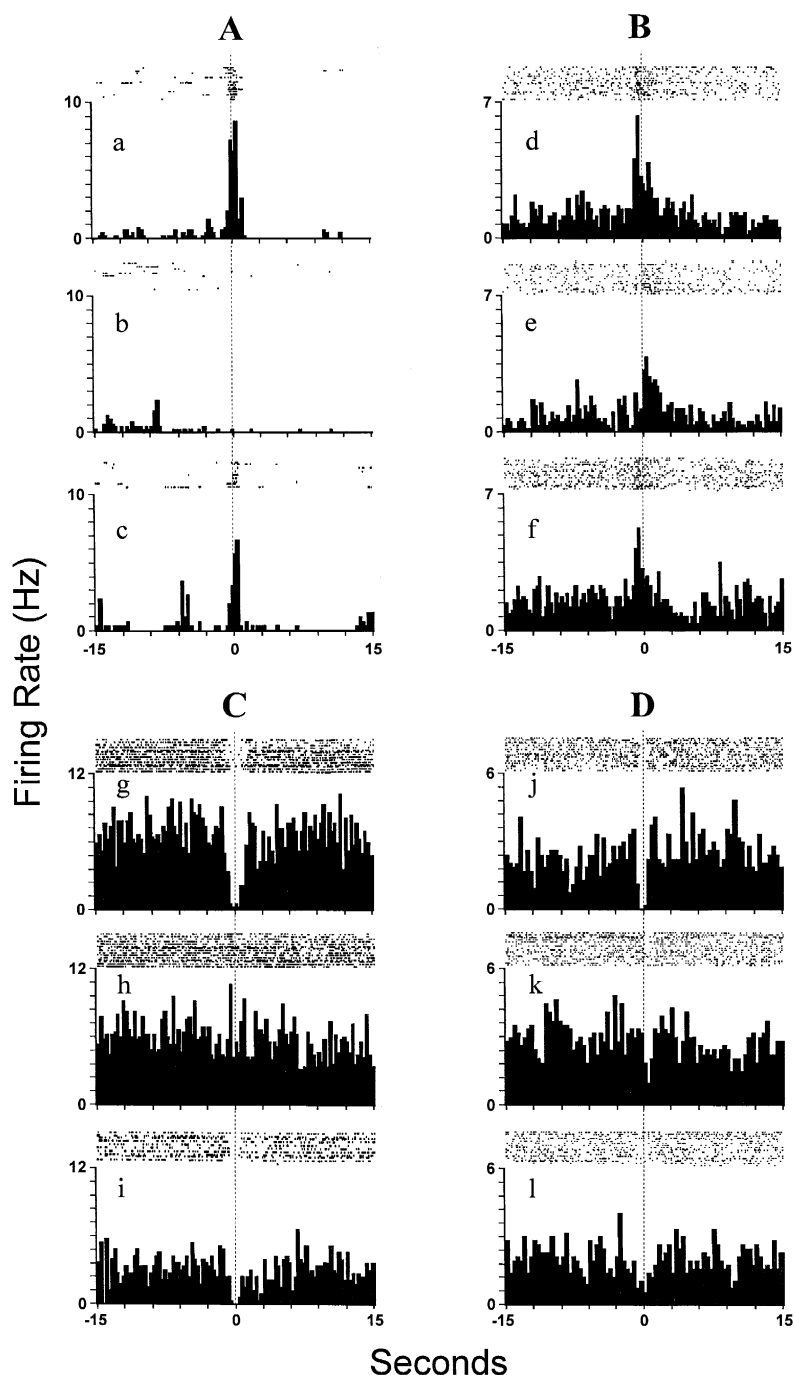


Fig. 2. Phasic firing that began before the onset of contingent infusions. Each panel (A–D) shows the firing patterns of a single neuron. In each panel, top-down, firing rate is plotted in relation to the following events (time zero): (1) response contingent infusions that were presented in conjunction with the tone and light stimulus events (a, d, g, j); (2) non-contingent infusions presented in conjunction with the tone and light stimulus events (b, e, h, k); and (3) non-reinforced lever presses (i.e., no cocaine infusion and no tone and light stimulus events) (c, f, i, l) during the non-contingent phase.

with that conclusion, all three types of firing patterns were present when the rat made non-reinforced presses during the non-contingent phases (i.e., when the press was present but the infusion was absent) (Fig. 2A_a vs. Fig. 2A_c; Fig. 2B_d vs. Fig. 2B_f; Fig. 2C_g vs. Fig. 2C_i; Fig. 2D_j vs. Fig. 2D_l).

Changes in firing rate that began after the operant was completed. During the contingent phase, 6 (21%) of the 29 responsive neurons showed an increase in firing that began within the first second post press (Fig. 3B_a vs. Fig. 3B_c) and typically terminated by 3 s post press (but for some neurons as late as 10 s post press). For about half of the neurons, the exclusively post-press firing pattern was completely absent when the operant was absent (Fig. 3A_a vs. Fig. 3A_b). For the other half of the neurons, the post-press firing was present, albeit diminished, when the operant was absent (Fig. 3B_c vs. Fig. 3B_d). These data show that for some neurons, the exclusively post-press firing had little or no relation to operant behavior. Consistent with this conclusion, exclusively post-press firing was diminished when the rat made non-reinforced presses during the non-contingent phases ($n = 1$) (Fig. 3B_c vs. Fig. 3B_e).

Topographical organization. Neurons from each of the three NAcc subterritories (core, shell, and rostral pole) [16] showed firing that was related to operant behavior. A larger sample will be required to assess whether there exist any subterritorial differences in the relative proportion of neurons that show firing related to the operant.

Summary. The within-session alternation between phases of contingent and non-contingent infusions systematically varied the amount of operant behavior that occurred before cocaine infusions while maintaining constant the recorded neuron, biological subject variables (e.g., sensitivity to exteroceptive stimuli, sensitivity to drug), and experiential subject variables (e.g., history of drug exposure, learning). Because the schedules of contingent and non-contingent infusions were identical, drug level at the time of infusion was also held constant. Hence, the behavioral and motivational state of the animal would also be expected to have been stable; this is especially true given that the schedule of infusion was determined by the animal. Consistent with these expectations, non-operant behavior was virtually identical during the contingent and non-contingent phases. Moreover, during the non-contingent phase, animals occasionally made lever presses and were therefore still motivated to self-infuse cocaine. Given the innocuous yet incisive and selective elimination of operant behavior during the seconds preceding non-contingent infusions, differences in firing patterns between the contingent and non-contingent phases are likely to be specifically related to the presence versus the absence of operant behavior.

With this in mind, the present data showed the following. All pre-press firing was related to the operant behavior, or to associated stimuli (under the present conditions and relative to infusion events); presence of the operant

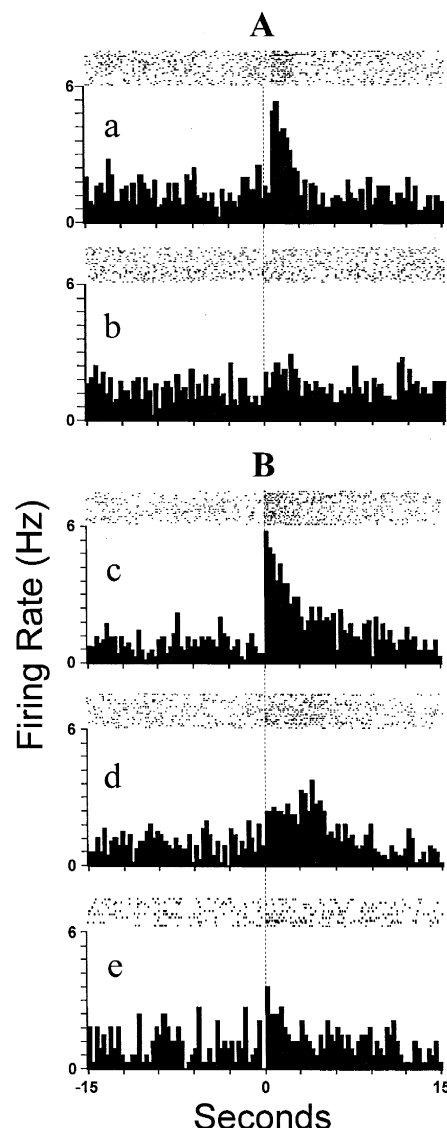


Fig. 3. Phasic firing that occurred exclusively after the onset of contingent cocaine infusions. Each panel (A and B) shows the firing pattern of a single neuron. For each panel, top-down, firing rate is plotted in relation to the following events (time zero): (1) response contingent infusions that were presented in conjunction with the tone and light stimulus events (a, c); and (2) non-contingent infusions that were presented in conjunction with the tone and light stimulus events (b, d). For Panel B firing rate is also displayed in relation to non-reinforced lever presses (i.e., no cocaine infusion and no tone and light stimuli) that were made during the non-contingent phase (e).

behavior was both sufficient and necessary for the firing to occur. Surprisingly, post-press firing of many neurons was also related to operant behavior. For the remaining neurons, post-press firing showed little or no relation to operant behavior and instead may have been responses to stimuli that were either coincident with the infusion or that indicated its imminent delivery.

The firing patterns in the contingent phase and the changes in those patterns during the non-contingent phase

are unlikely to reflect *execution* of motor behaviors. The comparable locomotion (to and from the lever) that occurs before and after each contingent infusion could not have engendered the asymmetric firing patterns [5,10]. Moreover, post-press firing that was present in relation to both contingent and non-contingent infusions occurred in conjunction with different types of motor behavior, i.e., locomotion (contingent phase) and focused stereotypy (non-contingent phase). Finally, most firing patterns persisted through a sequence of different behaviors that occurred in conjunction with the response contingent infusions (e.g., locomotion to the lever, the lever press, and then locomotion away from the lever).

Previous lesion studies showed that damage of NAcc neurons eliminates cocaine reinforced operant behavior [17]. The present data complement this finding by showing that elimination of the operant behavior diminished or eliminated the phasic firing exhibited by the majority of NAcc neurons. Both observations support the hypothesis that there is an important relationship between NAcc neurons and cocaine self-administration behavior.

Patterns similar in appearance to those studied here have been observed in the NAcc and striatum of monkeys [1,2] and rats [4] performing an operant maintained by delivery of a natural reinforcer. The studies conducted with monkeys showed that some firing patterns coincident with operant behavior were actually responses to stimuli that predicted reward availability. Moreover, some post-operant phasic firing patterns, observed most often in the NAcc and ventral striatum, were responses to delivery of the reward itself [1,2]. One interpretation of the present results is that NAcc neurons in the rat may respond to stimuli that are similarly related to the availability and delivery of *cocaine*. Although this interpretation needs to be corroborated using procedures that provide additional dissociations among instrumental behaviors, incentive stimuli, and reward stimuli (e.g., [1,2]), it is consistent with the known role that the NAcc plays in facilitating behavioral output controlled by stimulus-reward associations [3,14]. The interpretation is also consistent with hypotheses that stimulus-reward associations play an important role in drug addiction and that drugs of abuse facilitate drug taking behavior by affecting the same motivational brain circuitry that facilitates behavior controlled by natural reinforcers [11,12,14].

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References

- [1] P. Apicella, T. Lungberg, E. Scarnati, W. Schultz, Responses to reward in monkey dorsal and ventral striatum, *Exp. Brain Res.* 8 (1991) 491–500.
- [2] P. Apicella, E. Scarnati, T. Ljungberg, W. Schultz, Neuronal activity in monkey striatum related to the expectation of predictable environmental events, *J. Neurophysiol.* 68 (1992) 945–960.
- [3] M. Cador, T.W. Robbins, B.J. Everitt, H. Simon, M. LeMoal, L. Stinus, Limbic-striatal interactions in reward-related processes: modulation by the dopaminergic system, in: P. Willner, J. Scheel-Kruger (Eds.), *The Mesolimbic Dopamine System: from Motivation to Action*, Wiley, New York, 1991, pp. 225–250.
- [4] R.M. Carelli, S.A. Deadwyler, A comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats, *J. Neurosci.* 14 (1994) 7735–7746.
- [5] R.M. Carelli, V.C. King, R.E. Hampson, S.A. Deadwyler, Firing patterns of nucleus accumbens neurons during cocaine self-administration in rats, *Brain Res.* 626 (1993) 14–22.
- [6] J.-Y. Chang, S.F. Sawyer, R.-S. Lee, D.J. Woodward, Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats, *J. Neurosci.* 14 (1994) 1224–1244.
- [7] E. Fingl, D.M. Woodbury, General principles, in: L.S. Goodman, A. Gilman (Eds.), *The Pharmacological Basis of Therapeutics*, Macmillan Publishing Co., New York, 1975, p. 20.
- [8] Y.L. Hurd, J. Kehr, U. Ungerstedt, In vivo microdialysis as a technique to monitor drug transport: correlation of extracellular cocaine levels and dopamine overflow in the rat brain, *J. Neurochem.* 51 (1988) 1314–1316.
- [9] A.L. Misra, R.B. Pontani, S.J. Mule, [³H]Norcocaine and [³H]pseudococaine: effect of *n*-demethylation and C₂-epimerization of cocaine on its pharmacokinetics in the rat, *Experientia* 15 (1976) 895–897.
- [10] L.L. Peoples, M.O. West, Phasic firing of single neurons in the rat nucleus accumbens correlated with the timing of intravenous cocaine self-administration, *J. Neurosci.* 16 (1996) 3459–3473.
- [11] T.E. Robinson, K.C. Berridge, The neural basis of drug craving: an incentive-sensitization theory of addiction, *Brain Res. Rev.* 18 (1993) 247–291.
- [12] J. Stewart, H. De Wit, R. Eikelboom, Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants, *Psychol. Rev.* 91 (1984) 251–268.
- [13] A. Uzziak, M.O. West, L.L. Peoples, Phasic firing patterns recorded from individual nucleus accumbens neurons during cocaine appetitive behavior, in: *26th Annual Meeting of the Society for Neuroscience*, 1996, p. 927.
- [14] R.A. Wise, M.A. Bozarth, A psychomotor stimulant theory of addiction, *Psychol. Rev.* 94 (1987) 469–492.
- [15] R.A. Yokel, R. Pickens, Drug level of D- and L-amphetamine during intravenous self-administration, *Psychopharmacology (Berlin)* 34 (1974) 255–265.
- [16] D.S. Zahm, J.S. Brog, On the significance of subterritories in the “accumbens” part of the rat ventral striatum, *Neuroscience* 50 (1992) 751–767.
- [17] K.A. Zito, G. Vickers, D.C.S. Roberts, Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens, *Pharmacol. Biochem. Behav.* 23 (1985) 1029–1036.