

# Nicotine Self-Administration Acutely Activates Brain Reward Systems and Induces a Long-Lasting Increase in Reward Sensitivity

Paul J Kenny<sup>1</sup> and Athina Markou<sup>\*,1</sup>

<sup>1</sup>Department of Neuropsychopharmacology, The Scripps Research Institute, La Jolla, CA, USA

Nicotine is a major component of tobacco smoke contributing to the initiation and persistence of the harmful tobacco habit in human smokers. The reinforcing effects of nicotine likely arise through its ability to stimulate brain circuitry mediating the detection and experiencing of natural rewards. Nevertheless, remarkably little is known concerning the acute or long-lasting actions of nicotine on brain reward systems *in vivo*. Here, we investigated the effects of intravenously self-administered nicotine (0.03 mg/kg/infusion, free base) on the sensitivity of brain reward systems, reflected in alterations of intracranial self-stimulation (ICSS) thresholds in rats. Rats self-administered nicotine during 1 or 12 h daily sessions, with reward thresholds assessed 1 h before and 15 min after each self-administration session. Control rats remained nicotine naïve throughout. Nicotine self-administration increased the sensitivity of brain reward systems, detected by post-nicotine lowering of reward thresholds in 1 and 12 h rats. This nicotine-enhanced sensitivity of reward systems was reversed by the high-affinity nicotinic receptor antagonist dihydro- $\beta$ -erythroidine (DH $\beta$ E; 3 mg/kg). Surprisingly, nicotine-induced excitation of reward systems persisted for at least 36 days after nicotine self-administration had ceased. Overall, these data demonstrate that rats can voluntarily consume quantities of nicotine sufficient to increase the sensitivity of brain reward systems, an action likely crucial in establishing and maintaining the nicotine habit. Moreover, self-administered nicotine resets the sensitivity of reward systems to a new increased level, thereby imprinting an indelible 'memory' of its effects in reward systems, an action that so far appears unique to nicotine among drugs of abuse.

*Neuropsychopharmacology* (2006) **31**, 1203–1211. doi:10.1038/sj.npp.1300905; published online 28 September 2005

**Keywords:** nicotine; self-administration; intracranial self-stimulation; reward; addiction

## INTRODUCTION

It is generally accepted that tobacco-smoking behavior is at least partly a manifestation of nicotine addiction, and that nicotine is one of the most powerfully addictive drugs consumed by humans. The reinforcing actions of nicotine and other major drugs of abuse arise through their shared ability to usurp brain circuitry responsible for sensitivity to, learning about, and experiencing of natural rewards (Wise, 1996; Rice and Cragg, 2004). Moreover, adaptations that occur in brain reward circuits in response to their excessive, nonphysiological stimulation by drugs of abuse may contribute to the development of the compulsive drug use that characterizes addiction (Ahmed *et al*, 2002; Kenny *et al*, 2003). Hence, an understanding of the actions of nicotine

on the activity of brain reward systems may illuminate mechanisms that drive nicotine addiction in human tobacco smokers. Nevertheless, remarkably little is known about the acute or long-lasting actions of nicotine on brain reward pathways.

The intracranial self-stimulation (ICSS) procedure provides an accurate measure of the sensitivity of brain reward systems *in vivo* (Kornetsky *et al*, 1979). In the ICSS procedure, rats respond vigorously to obtain rewarding electrical self-stimulation via a stimulating electrode implanted in various components of the brain's reward systems (Olds and Milner, 1954). By bypassing much of the input side of conventional natural reinforcers (feeding circuits, etc.) and directly activating brain reward systems, ICSS provides a unique tool to investigate the direct influence of drugs of abuse on brain reward systems. Indeed, rats demonstrate little satiation to ICSS during extended testing sessions (Olds, 1958). Repeated exposure to ICSS over days does not alter the baseline activity of brain reward systems (Annau *et al*, 1974), and prior exposure to ICSS does not alter patterns of intravenous drug self-administration (Markou and Koob, 1991; Ahmed *et al*,

\*Correspondence: Dr A Markou, Department of Neuropsychopharmacology, CVN-7, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA, Tel: +1 858 784 7244, Fax: +1 858 784 7405, E-mail: amarkou@scripps.edu  
Received 3 May 2005; revised 19 July 2005; accepted 5 August 2005  
Online publication: 22 August 2005 at <http://www.acnp.org/citations/Npp082205050281/default.pdf>

2002; Kenny *et al.*, 2003). As such, ICSS is particularly useful for monitoring the effects of abuse drugs on brain reward systems across extended periods of time.

The minimal stimulation intensity that maintains ICSS behavior is termed the reward threshold (Kornetsky *et al.*, 1979; Markou and Koob, 1992). Previously, experimenter-administered or self-administered infusions of various drugs of abuse such as psychomotor stimulants (Wise *et al.*, 1977; Bauco and Wise, 1997; Kenny *et al.*, 2003) and opiates (Bespalov *et al.*, 1994) were shown to lower the reward threshold, indicating increased sensitivity to the rewarding effects of ICSS that likely arises through drug-induced amplification of reward signals in the brain. Here, we directly investigated the short- and long-term actions of nicotine consumption on the sensitivity of brain reward systems *in vivo* by monitoring ICSS thresholds in rats with daily access to intravenous nicotine self-administration.

## MATERIALS AND METHODS

### Subjects

Subjects were 35 male Wistar rats weighing 300–320 g upon arrival at the laboratory. Rats were obtained from Charles River Laboratories (Raleigh, NC) and were housed in groups of two or three per cage. Animals were maintained in a temperature-controlled vivarium under a 12 h light/dark cycle, with *ad libitum* access to food and water until ICSS training commenced. Animals received their daily nicotine self-administration session during the dark portion of the light/dark cycle. Animals had ICSS thresholds assessed at time points according to the experimental design. All animals were treated in accordance with the guidelines of the National Institutes of Health regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care.

### Apparatus

ICSS training and testing took place in 20 Plexiglas operant chambers ( $25 \times 31 \times 24 \text{ cm}^3$ ; Med Associates, St Albans, VT). The floors of the operant chambers were constructed of parallel aluminum rods spaced 1.25 cm apart. One wall contained a metal wheel manipulandum that required 0.2 N force to rotate it one-quarter of a turn. The wheel (5 cm in width) extended  $\sim 3 \text{ cm}$  out of the wall. Each testing chamber was enclosed within a light- and sound-attenuated chamber ( $62 \times 63 \times 43 \text{ cm}^3$ ). Intracranial stimulation was delivered by constant current stimulators (Stimtech model 1200; San Diego Instruments, San Diego, CA). Subjects were connected to the stimulation circuit through flexible bipolar leads (Plastics One, Roanoke, VA) attached to gold-contact swivel commutators (model SL2C; Plastics One) mounted above the chamber. The stimulation parameters, data collection, and all test session functions were controlled by a microcomputer. Nicotine self-administration took place in 15 home-made metal and Plexiglas, sound-attenuated operant chambers ( $29 \times 24 \times 19.5 \text{ cm}^3$ ) with a floor constructed of aluminum rods spaced 1.3 cm apart. In each chamber, one wall contained two metal retractable levers, one 'active' and one 'inactive', that were mounted

2.5 cm above the floor and required a 0.1 N force to be pressed. Plastic swivels (Plastics One Co., Roanoke, VA) connected the animals to syringes operated by Razel pumps that delivered the drug. Data collection and all programming functions were controlled by an IBM-compatible microcomputer.

### Surgery

Rats were anesthetized by inhalation of 1–3% isoflurane in oxygen and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The incisor bar was adjusted to 5 mm above the interaural line, and the skull was exposed. Stainless-steel bipolar electrodes (11 mm in length) were implanted into the posterior lateral hypothalamus (AP:  $-0.5 \text{ mm}$  from bregma; ML:  $\pm 1.7 \text{ mm}$  from midline; DV:  $8.3 \text{ mm}$  from dura) according to the atlas of Pellegrino *et al.* (1979). Four indentations were made in the skull to accommodate screws that, together with the application of dental acrylic, held the electrode in place. All rats were then prepared with catheters in the jugular vein as described previously (Caine and Koob, 1993). The catheter was passed subcutaneously to a polyethylene assembly mounted on the animal's back. This assembly consisted of a guide cannula (Plastics One Co., Roanoke, VA) attached to a  $4 \text{ cm}^2$  piece of marlex mesh with epoxy. The marlex mesh was placed under the skin on the animal's back.

### Procedure

**ICSS threshold.** All rats (nicotine self-administering and control rats) were food restricted throughout the experiment to maintain them at 85% of their normal body weight obtained with free-feeding conditions. Rats were trained to respond according to a modification of the discrete-trial current-threshold procedure of Kornetsky and Esposito (1979), previously described in detail (Markou and Koob, 1992). Briefly, a trial was initiated by the delivery of a noncontingent electrical stimulus. This electrical reinforcer had a train duration of 500 ms and consisted of 0.1 ms rectangular cathodal pulses that were delivered at a frequency of 50–100 Hz. The frequency of the stimulation was selected for individual animals so that current-intensity thresholds of each subject were within 50–200  $\mu\text{A}$ , and thus allowed both threshold elevation or lowering to be detected. This frequency was held constant throughout the experiment. A one-quarter turn of the wheel manipulandum within 7.5 s of the delivery of the noncontingent electrical stimulation resulted in the delivery of an electrical stimulus identical in all parameters to the noncontingent stimulus that initiated the trial. After a variable intertrial interval (7.5–12.5 s, average of 10 s), another trial was initiated with the delivery of a noncontingent electrical stimulus. Failure to respond to the noncontingent stimulus within 7.5 s resulted in the onset of the intertrial interval. Responding during the intertrial interval delayed the onset of the next trial by 12.5 s. Current levels were varied in alternating descending and ascending series. A set of three trials was presented for each current-intensity. Current intensities were altered in 5  $\mu\text{A}$  steps. In each testing session, four alternating descending-ascending series were presented. The threshold for each series was defined as the midpoint

between two consecutive current intensities that yielded 'positive scores' (animals responded for at least two of the three trials) and two consecutive current intensities that yielded 'negative scores' (animals did not respond for two or more of the three trials). The overall threshold of the session was defined as the mean of the thresholds for the four individual series. Each testing session was ~30 min in duration.

**Intravenous nicotine self-administration.** All rats were trained to press an active lever for 45 mg food pellets on a fixed-ratio 5 (FR5) time-out 20 s (TO20) schedule of reinforcement. Rats were also presented with an inactive lever during training sessions, responses on which were recorded but were without scheduled consequence. Rats were trained until stable responding for food was obtained, defined as >90 pellets per 1 h session. 1 and 12 h rats responded for nicotine on an FR5TO20 s schedule of reinforcement, when five responses on the active lever resulted in the delivery of one nicotine injection (0.03 mg/kg/infusion dissolved in 0.1 ml of sterile 0.9% sterile saline, free base) over 1 s duration, and initiated a 20 s time-out (TO) period signaled by a light cue located above the lever, during which responding on the lever was without consequence. Responding on the inactive lever during nicotine self-administration sessions was again recorded but was without scheduled consequence (data not presented).

### Experimental Procedure

Rats were trained in the ICSS threshold procedure as described above once daily until stable reward thresholds were established, defined as  $\leq 10\%$  variation in thresholds for three consecutive days and requiring between 7 and 10 days. After the establishment of stable baseline thresholds, all rats had reward thresholds assessed twice daily for five consecutive days immediately prior to their first nicotine self-administration session to habituate them to multiple daily ICSS threshold assessments. On the two days immediately prior to their first nicotine self-administration session, rats were trained to lever press for food reinforcement as described above between the two daily ICSS threshold assessments, in order to accelerate acquisition of the lever press response for nicotine. Rats were then permitted to respond for intravenous nicotine infusions during 20 consecutive daily 1 h (1 h rats;  $n = 7$ ) or 12 h (12 h rats;  $n = 8$ ) self-administration sessions. Rats in 12 h sessions also had access to water during self-administration sessions.

Rats had reward thresholds assessed 1 h before (pre-thresholds) and 15-min after (post-thresholds) each daily nicotine self-administration session. Control rats ( $n = 5$ ) remained nicotine naïve over the duration of the entire experiment and had thresholds assessed at time points similar to 12 h rats. To assess the role of high-affinity neuronal nicotinic acetylcholine receptor (nAChRs) in regulating the stimulatory actions of nicotine on brain reward systems, the effects of the high-affinity neuronal nAChR antagonist dihydro- $\beta$ -erythroidine (DH $\beta$ E) on nicotine-induced lowering of reward thresholds were examined. Control, 1, and 12 h rats were injected with

DH $\beta$ E (3 mg/kg, salt) immediately after post-threshold assessment on day 15 of nicotine self-administration, and reward thresholds were immediately assessed for the third time that day. For comparison, rats were injected with saline immediately after post-threshold assessment on day 20, and reward thresholds were assessed.

After 20 consecutive days of access to nicotine self-administration, rats were no longer permitted to self-administer nicotine and remained in their home cages for the remainder of the experiment except for ICSS threshold assessment. Thresholds were assessed in the three groups of rats up to 8 days after the final nicotine self-administration session. As reward thresholds were unexpectedly lowered throughout the post-nicotine phase of the experiment in 1 and 12 h rats, we examined whether this effect could be replicated in a second cohort of control ( $n = 7$ ), 1 h ( $n = 4$ ), and 12 h ( $n = 4$ ) rats. This second cohort of rats underwent the same experimental procedure outlined above for the first cohort of animals, with the exception that post-nicotine reward thresholds were assessed until 36 days in these rats. Only the data from the post-nicotine phase of the experiment are presented here for this second cohort of rats (Figure 4). Importantly, however, data collected during the nicotine self-administration phase in this second cohort of animals closely replicated the findings reported below.

### Statistical Analyses

The number of nicotine infusions earned by 1 and 12 h rats during the 20 days of access to nicotine self-administration was analyzed by two-way repeated-measures analysis of variance (ANOVA), with *Access* (1 or 12 h) as a between-subjects factor and *Days* as a within-subjects repeated-measures factor. For ICSS data, percentage change from baseline reward thresholds was calculated by expressing the reward threshold scores during the nicotine self-administration phase of the experiment (including the assessment of the effects of DH $\beta$ E), and the post-nicotine phase of the experiment as a percentage of baseline thresholds, minus 100. The baseline reward thresholds were the mean thresholds obtained for each rat on the five ICSS sessions prior to the first nicotine self-administration session. During the nicotine self-administration phase of the experiment, threshold scores for control, 1, or 12 h rats were analyzed with three-factor repeated measures ANOVA, with *Session* (pre-thresholds or post-thresholds) as a within-subjects factor, *Days* as a within-subjects repeated-measures factor, and *Access* (control, 1 or 12 h) as a between-subjects factor. This analysis was followed by individual two-factor repeated-measures ANOVAs for control, 1, and 12 h rats, with *Session* (pre-thresholds or post-thresholds) as a within-subjects factor and *Days* as a within-subjects repeated-measures factor. To investigate the effects of DH $\beta$ E on nicotine-induced lowering of reward thresholds, pre-thresholds, post-thresholds, and post-DH $\beta$ E thresholds on day 15, and pre-thresholds, post-thresholds, and post-saline thresholds on day 20, for control, 1, and 12 h rats were analyzed by three-way repeated-measures ANOVA. Factors for this analysis were *Access* (control, 1 or 12 h) as a between-subjects factor, *Drug* (saline or DH $\beta$ E) as a within-subjects factor, and *Session* (pre-thresholds, post-thresholds, and

post-DH $\beta$ E/saline thresholds) as a within-subjects repeated-measures factor. Reward thresholds for control, 1 and 12 h rats during the post-nicotine phase of the experiment were analyzed by two-factor repeated-measures ANOVA, with prior *Access* as a between-subjects factor and *Days* as a within-subjects repeated-measures factor. After statistically significant effects in the ANOVAs, *post hoc* comparisons among means were conducted with Fisher's LSD test. The level of significance was set at 0.05.

## RESULTS

### Nicotine Self-Administration in Rats with Restricted or Extended Access to Nicotine

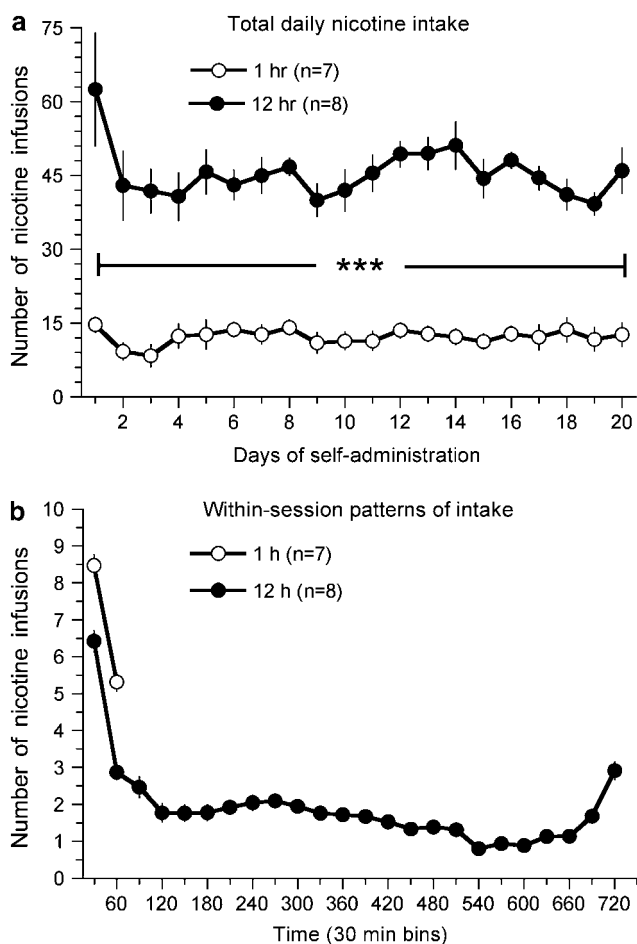
Stable patterns of daily nicotine intake were rapidly established in rats with 1 or 12 h daily access to nicotine self-administration (Figure 1a). As expected, rats with 12 h

daily access to nicotine consumed greater quantities of nicotine ( $\sim 1.36$  mg/kg/day) than rats with 1 h daily access ( $\sim 0.38$  mg/kg/day) (Figure 1a; main effects of *Access* ( $F_{(1,13)} = 154.9$ ,  $p < 0.001$ ), *Days* ( $F_{(13,19)} = 3.5$ ,  $p < 0.001$ ), and *Access*  $\times$  *Days* interaction ( $F_{(19,247)} = 3.0$ ,  $p < 0.001$ ). In addition to stable daily nicotine intake, 1 and 12 h rats also established stable patterns of within-session nicotine intake, as reflected in the number of nicotine infusions earned during 30 min bins in each self-administration session over the entire 20 sessions (Figure 1b). Intake in 1 h rats was characterized by a 'loading' phase during the first 30 min of the 1 h session, with less nicotine being consumed during the final 30 min of each session (Figure 1b). In 12 h rats, intake was characterized by an initial loading phase during the first 30 min; this was followed by a 'maintenance' phase where rats self-administered between one and two infusions every 30 min for approximately 10 h; finally, nicotine intake increased during the last 30 min of each 12 h session, when rats consumed approximately three infusions (Figure 1b).

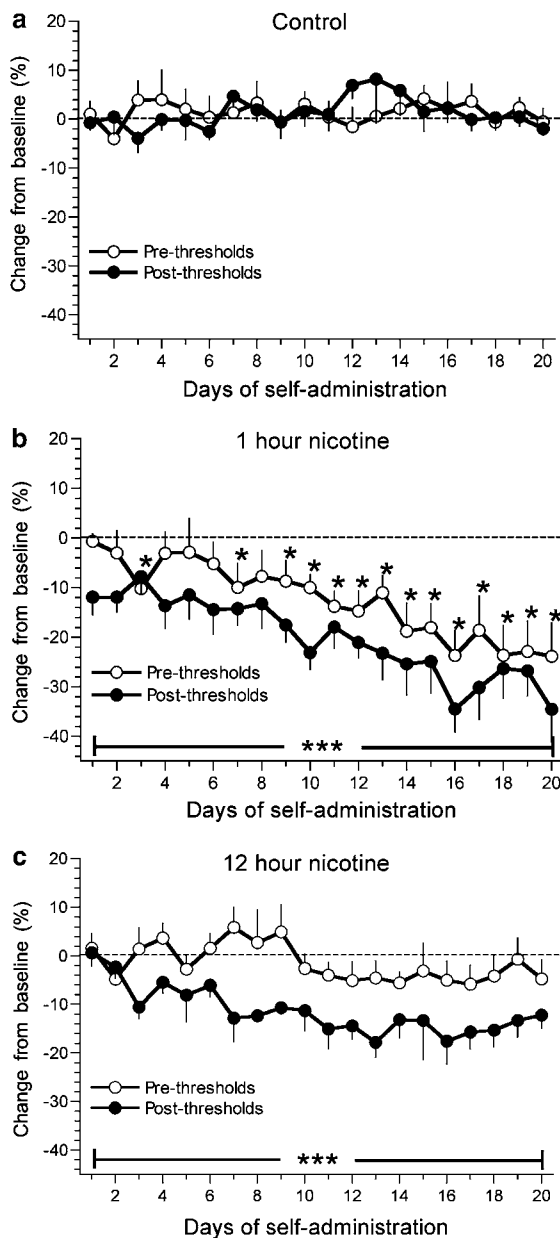
### Nicotine Self-Administration Increases the Sensitivity of Brain Reward Systems

Mean absolute baseline reward thresholds in control, 1, and 12 h rats were  $116.4 \pm 12.0$ ,  $105.9 \pm 5.9$ , and  $113.5 \pm 13.1$   $\mu$ A, respectively. Self-administration of nicotine significantly lowered post-thresholds in 1 and 12 h rats, reflected in a statistically significant effect of *Access* ( $F_{(2,17)} = 9.3$ ,  $p < 0.01$ ), *Session* ( $F_{(1,17)} = 59.8$ ,  $p < 0.001$ ), *Days* ( $F_{(19,323)} = 4.8$ ,  $p < 0.001$ ), and a significant *Access*  $\times$  *Days*  $\times$  *Session* interaction effect ( $F_{(38,323)} = 1.6$ ,  $p < 0.05$ ). Further analysis of the main effect of *Session* demonstrated that reward thresholds were significantly lowered in 1 h rats compared with control ( $p < 0.001$ ) and 12 h rats ( $p < 0.05$ ).

To further analyze the effects of nicotine on thresholds, individual two-factor ANOVAs were carried out for control, 1, and 12 h rats. Reward thresholds remained stable and unaltered over the duration of the entire experiment in control rats at both daily time points of assessment (Figure 2a) (*Session* ( $F_{(1,4)} = 0.01$ , NS); *Days* ( $F_{(19,76)} = 0.5$ , NS); *Session*  $\times$  *Days* interaction ( $F_{(19,76)} = 1.1$ , NS)). In contrast, nicotine self-administration lowered post-thresholds in 1 h rats (Figure 2b), reflected in statistically significant effects of *Session* ( $F_{(1,6)} = 39.8$ ,  $p < 0.001$ ) and *Days* ( $F_{(19,114)} = 8.9$ ,  $p < 0.001$ ) (Figure 2b). There was a marked downward shift in daily pre-thresholds and post-thresholds in 1 h rats that progressively increased in magnitude as days of exposure to nicotine increased (Figure 2b). Analysis of pre-thresholds over *Days* from 1 h rats demonstrated a significant lowering of pre-thresholds as exposure to nicotine increased ( $F_{(19,114)} = 7.3$ ,  $p < 0.001$ ); pre-thresholds were significantly lowered on days 3, 7, and 9–20 compared with pre-thresholds obtained on day 1 (Figure 2b). Nicotine self-administration significantly lowered post-thresholds in 12 h rats (Figure 2c), reflected in statistically significant effects of *Session* ( $F_{(1,7)} = 53.5$ ,  $p < 0.001$ ) and *Days* ( $F_{(19,133)} = 2.0$ ,  $p < 0.05$ ). In contrast to 1 h rats, however, pre-nicotine thresholds remained stable and unaltered over *Days* in the 12 h rats ( $F_{(19,133)} = 1.0$ , NS; Figure 2c).



**Figure 1** Nicotine self-administration increases the sensitivity of brain reward systems. (a) Daily nicotine intake in rats with limited (1 h) or extended (12 h) daily access to nicotine self-administration. Mean number of nicotine infusions ( $\pm$  SEM) earned each day by rats with 1 or 12 h daily access to nicotine (0.03 mg/kg/infusion; free-base). \*\*\* $p < 0.001$ , main effect of access (1 or 12 h) on the nicotine doses consumed, two-way repeated measures ANOVA. (b) Within-session number of nicotine infusions earned over the entire 20 days of access to nicotine. Mean infusions ( $\pm$  SEM) earned by 1 and 12 h rats are presented in 30 min time bins.



**Figure 2** Nicotine self-administration increases the sensitivity of brain reward systems. (a) Daily pre-nicotine and post-nicotine reward thresholds ( $\pm$  SEM) remained stable and unaltered in nicotine-naïve control rats. (b) Daily post-thresholds ( $\pm$  SEM), assessed 15 min after each daily nicotine self-administration session, were lowered compared with pre-thresholds ( $\pm$  SEM), assessed 1 h before each self-administration session, in 1 h ( $n=7$ ) rats. \*\*\* $p<0.001$ , main effect of nicotine on reward thresholds, two-way repeated measures ANOVA. In addition, there was a progressive lowering of pre-thresholds in 1 h rats that resulted in a gradual downward shift in both pre-thresholds and post-thresholds. \* $p<0.05$ , compared with pre-thresholds obtained prior to the first nicotine self-administration session (day 1), post-hoc test after one-way ANOVA. (c) Daily post-thresholds ( $\pm$  SEM) were lowered compared with pre-nicotine thresholds ( $\pm$  SEM) in 12 h ( $n=8$ ) rats. \*\*\* $p<0.001$ , main effect of nicotine on reward thresholds, two-way repeated measures ANOVA.

### High-Affinity Nicotinic Receptors Regulate Nicotine's Actions on Brain Reward Systems

The high-affinity nAChR antagonist DH $\beta$ E, but not saline, reversed the lowering of post-thresholds in 1 and 12 h rats

induced by self-administered nicotine, reflected in a statistically significant effect of *Access* ( $F_{(2,17)}=7.9$ ,  $p<0.01$ ), *Drug* ( $F_{(1,2)}=8.2$ ,  $p<0.05$ ), and *Session* ( $F_{(2,4)}=21.6$ ,  $p<0.001$ ). An *Access*  $\times$  *Days*  $\times$  *Session* interaction effect approaching significance was also observed ( $F_{(38,323)}=2.3$ ,  $p=0.078$ , NS) (Figure 2). In keeping with our hypothesis that DH $\beta$ E would block the effects of nicotine on thresholds, *post hoc* analyses demonstrated that post-thresholds were significantly lowered compared with pre-thresholds in 1 h ( $p<0.05$ ) and 12 h rats ( $p<0.01$ ) on days 15 and 20, while DH $\beta$ E administration on day 15, but not saline on day 20, attenuated this nicotine-induced lowering of reward thresholds (Figure 3).

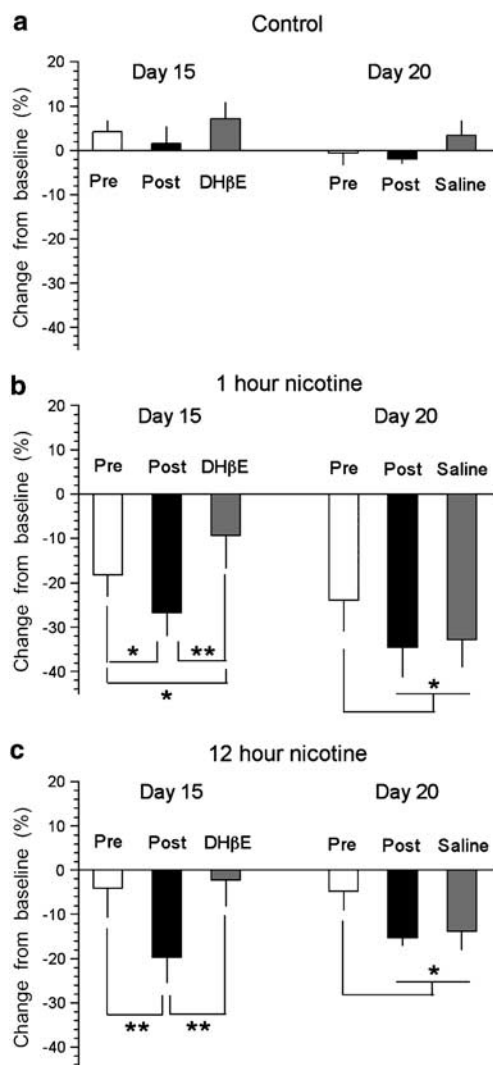
### Nicotine Induces a Persistent Increase in the Sensitivity of Brain Reward Systems

Two-way repeated-measures ANOVA demonstrated a main effect of prior nicotine access on reward thresholds during the post-nicotine phase of the experiment, reflected in a significant effect of *Access* ( $F_{(2,17)}=6.0$ ,  $p<0.01$ ), no effect of *Days* ( $F_{(8,16)}=1.5$ , NS), and no significant *Access*  $\times$  *Days* interaction effect ( $F_{(16,136)}=1.2$ , NS). Test of the simple main effect of *Access* demonstrated that during the post-nicotine phase of the experiment, reward thresholds of 1 h and 12 h rats were lowered compared with control rats ( $p<0.01$ ;  $p<0.05$ , respectively), and the magnitude of this effect was similar in 1 and 12 h rats ( $p=0.14$ , N.S.) (Figure 4).

To replicate the unexpected observation that reward thresholds were lowered throughout the post-nicotine phase of the experiment in 1 and 12 h rats, and to examine the persistence of this phenomenon, reward thresholds were assessed in a second cohort of control, 1, and 12 h rats. Reward thresholds were assessed in this second set of rats for 36 days after their final nicotine self-administration session. Analysis of post-nicotine reward thresholds from these subjects again demonstrated a significant effect of prior access to nicotine, reflected in a significant effect of *Access* ( $F_{(2,12)}=7.9$ ,  $p<0.01$ ), no effect of *Days* ( $F_{(19,38)}=0.7$ , NS), and no significant *Access*  $\times$  *Days* interaction effect ( $F_{(38,228)}=0.6$ , NS). Test of the simple main effect of *Access* demonstrated that reward thresholds remained significantly lowered in 1 ( $p<0.05$ ) and 12 h ( $p<0.01$ ) rats during the entire 36-day post-nicotine period compared with control rats (Figure 5).

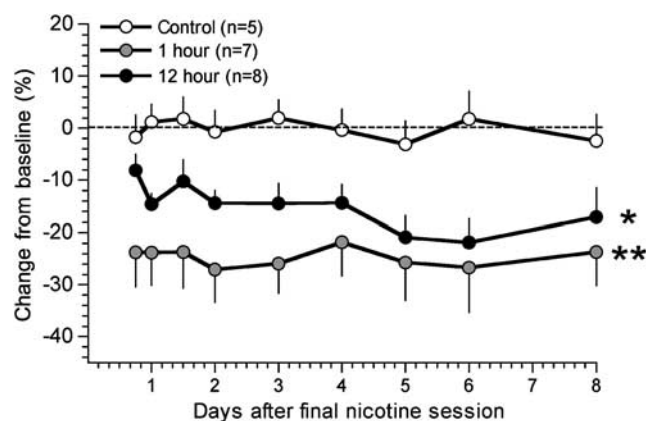
### DISCUSSION

Similar to other major drugs of abuse, experimenter-administered bolus nicotine injections increase the sensitivity of brain reward systems, reflected in lowered brain reward thresholds (Harrison *et al.*, 2002). Here, we show that rats volitionally consume quantities of nicotine during 1 or 12 h self-administration sessions sufficient to increase the sensitivity of brain reward systems, and thereby amplify the rewarding effects of ICSS. Thus, achieving maximal increases in the sensitivity of brain reward systems may be a crucial factor in determining the amount of nicotine self-administered, and its pattern of intake. Unexpectedly, however, the stimulatory action of nicotine on reward

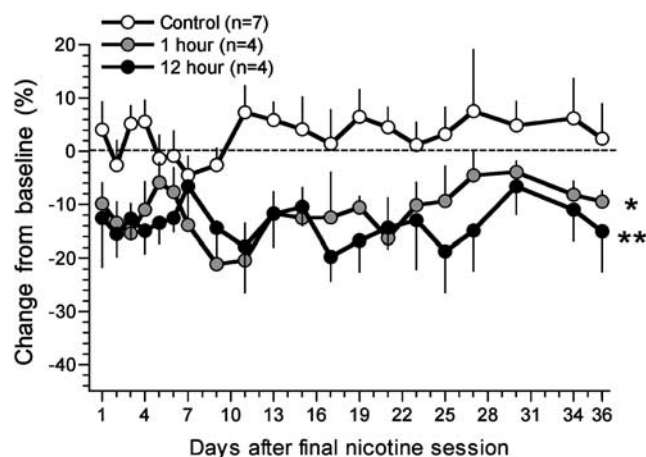


**Figure 3** The competitive nicotinic receptor antagonist DHβE reversed the increased sensitivity of brain reward systems induced by self-administered nicotine. Pre-thresholds (Pre), post-thresholds (post), and post-DHβE thresholds (DHβE) or post-saline thresholds (saline) were assessed on days 15 and 20 of nicotine self-administration. (a) Reward thresholds ( $\pm$ SEM) remained stable and unaltered in nicotine-naïve control rats. (b) Injection of DHβE reversed the lowering of post-thresholds compared with pre-thresholds observed on day 15 (left panel) or saline on day 20 (right panel) in nicotine-naïve control rats. (c) Injection of DHβE reversed the lowering of post-thresholds compared with pre-thresholds observed on day 15 (left panel) in 1 h rats. Saline injection did not reverse the lowering of post-thresholds compared with pre-thresholds observed on day 20 (right panel) in 1 h rats.  $*p < 0.05$ ,  $**p < 0.01$ ,  $post\ hoc$  test after three-way ANOVA. (c) Injection of DHβE reversed the lowering of post-thresholds compared with pre-thresholds on day 15 (left panel) in 12 h rats. Saline injection did not reverse the lowering of post-thresholds compared with pre-thresholds on day 20 (right panel) in 12 h rats.  $*p < 0.05$ ,  $**p < 0.01$ ,  $post\ hoc$  test after three-way ANOVA.

systems was very long lasting, as reward thresholds remained persistently lowered in 1 and 12 h rats during the entire post-nicotine assessment period when rats no longer had access to nicotine self-administration. Thus, self-administered nicotine induces a persistent amplification in the brain's sensitivity to rewarding stimuli, an action of nicotine that appears fundamentally different from the long-term actions of other drugs of abuse such as cocaine (Markou and Koob, 1991; Ahmed *et al*, 2002; Kenny *et al*, 2003) or heroin (Kenny, Chen, Markou, and Koob,



**Figure 4** Nicotine re-sets baseline reward sensitivity to a new amplified level. Reward thresholds were assessed 0.75, 1, 1.5, 2, 3, 4, 5, 6, and 8 days after the final nicotine self-administration session. Reward thresholds ( $\pm$ SEM) remained stable and unaltered in nicotine-naïve control rats. Reward thresholds ( $\pm$ SEM) remained persistently lowered in 1 h rats ( $n=7$ ) during this post-nicotine period.  $**p < 0.01$  compared with thresholds from control rats, test of simple main effect. In 12 h rats, reward thresholds ( $\pm$ SEM) rapidly lowered during the post-nicotine period until they reached a level below baseline similar to that observed in 1 h rats.  $*p < 0.05$  compared with thresholds from control rats, test of simple main effect.



**Figure 5** Nicotine-induced increases in reward sensitivity are very long lasting. Reward thresholds were assessed in a second cohort of control, 1, and 12 h rats until 36 days after the final nicotine self-administration session. Reward thresholds ( $\pm$ SEM) remained stable and unaltered in nicotine-naïve control rats. Reward thresholds ( $\pm$ SEM) remained persistently lowered in 1 and 12 h rats during this post-nicotine period.  $*p < 0.05$  1 h rats compared with control rats,  $**p < 0.01$  12 h rats compared with control rats, test of simple main effects.

unpublished observations) that usually results in long-term compensatory decreases in the activity of brain reward systems during the early abstinence period.

The present data may account for some of the unusual properties of nicotine compared with other drugs of abuse. For example, extended access to cocaine (Ahmed and Koob, 1998; Ahmed *et al*, 2002) or morphine (Ahmed *et al*, 2000) self-administration results in a progressive 'escalation' in drug intake reminiscent of the compulsively increased drug intake in human drug addicts, an effect not observed with

extended access to nicotine (Figure 1a). Moreover, schedules of access to cocaine and other drugs of abuse that result in escalated drug intake also result in decreases of reward sensitivity, which presumably arise to counter the reward-facilitating actions of these drugs (Ahmed *et al*, 2002; see also Markou and Koob, 1991; Kenny *et al*, 2003). Thus, escalated intake of cocaine and other addictive drugs likely develops to counter progressive reward deficits associated with extended drug access. The observations above suggest that nicotine consumption does not escalate over time because a similar progressive reward deficit does not develop with extended periods of nicotine self-administration, at least not under the present conditions.

It is interesting to note that spontaneous or nAChR antagonist-precipitated withdrawal from nicotine delivered via chronic indwelling subcutaneous osmotic mini-pumps, as opposed to self-administration, induced elevations of reward thresholds above pre-nicotine baseline levels similar to the threshold elevations observed after cessation of cocaine self-administration (Epping-Jordan *et al*, 1998; Watkins *et al*, 2000). Thus, volitional nicotine consumption may induce adaptations in reward circuitries different from those produced by passively administered nicotine, or self-administration of other drugs of abuse. Consistent with this hypothesis, nicotine induced long-term potentiation of excitatory inputs to reward areas in the rat brain that persisted long after nicotine was no longer present (Mansvelder and McGehee, 2000). It is important to note, however, that the amounts of nicotine self-administered during the 1 or 12 h sessions in the present studies (approximately 0.38 and 1.36 mg/kg nicotine free base per day, respectively) were significantly less than the daily amounts delivered by a mini-pump in the studies described above (3.16 mg/kg nicotine per day). Thus, it is possible that access to nicotine self-administration for durations longer than the 20 days reported here may induce further adaptations in brain reward systems similar to those observed in rats treated with nicotine via mini-pumps, and in cocaine self-administering rats. Nevertheless, repeated 1 or 6 h daily access to nicotine self-administration was recently shown to induce 'physical' dependence on nicotine, such that a spontaneous somatic withdrawal syndrome was observed 23 h after cessation of nicotine intake (Paterson and Markou, 2004). Thus, although somatic signs of spontaneous withdrawal were not investigated in the present study, it is likely that 'physical' dependence on nicotine developed in parallel with the reward hypersensitivity reported here. Overall, the present findings offer the intriguing possibility that volitional nicotine consumption induces long-lasting adaptations in reward pathways fundamentally different from those produced by other drugs of abuse such as cocaine or by higher doses of passively experimenter-administered nicotine. However, to fully explore the above hypothesis it will be necessary to examine the sensitivity of brain reward systems in 'yoked' control rats that passively receive a pattern of nicotine infusions similar to that observed in rats that volitionally self-administer nicotine infusions.

Accumulating evidence suggests that  $\alpha 4$ - and  $\beta 2$ -containing nAChRs may play a crucial role in regulating the stimulatory effects of nicotine on brain reward systems. For example, similar rates of intravenous cocaine self-

administration were observed in wild-type and mutant mice in which the  $\beta 2$  nAChR subunit had been null mutated ( $\beta 2^{-/-}$  mice). However, when cocaine was substituted with nicotine, wild-type mice persisted in responding to nicotine, whereas  $\beta 2^{-/-}$  mice rapidly extinguished responding (Picciotto *et al*, 1998; Epping-Jordan *et al*, 1999). Most recently,  $\alpha 4$  knock-in mice, bearing a mutation that rendered  $\alpha 4$ -containing receptors hypersensitive to nicotine, demonstrated a nicotine-induced conditioned place preference at nicotine doses 50-fold lower than those necessary to induce a place preference in wild-type mice (Tapper *et al*, 2004). Overall, these observations provide strong evidence that  $\alpha 4$ - and  $\beta 2$ -containing nAChRs mediate the effects of nicotine on brain reward systems (Picciotto *et al*, 1997; Tapper *et al*, 2004). In the present studies, we show that DH $\beta$ E reversed nicotine-induced lowering of reward thresholds. DH $\beta$ E is considered relatively selective for  $\alpha 4\beta 2$  high-affinity nAChRs (Harvey and Luetje, 1996; Harvey *et al*, 1996), although the selectivity of DH $\beta$ E and other nAChR antagonists for specific nAChR subtypes is poor (Damaj *et al*, 1995; Harvey and Luetje, 1996; Harvey *et al*, 1996). Nevertheless, these data are consistent with an important role for high-affinity, possibly  $\alpha 4\beta 2$ , nAChRs in regulating the stimulatory actions of self-administered nicotine on brain reward systems.

In the present studies, we observed a progressive downward shift in baseline reward thresholds in 1 h rats during the 20 days of self-administration, suggesting that this schedule of access to nicotine induced a progressively greater increase in the sensitivity of brain reward systems. Curiously, a similar downward shift of reward thresholds did not emerge in 12 h rats during the nicotine self-administration phase of the experiment (Figure 2c). Importantly, however, reward thresholds were similarly lowered in 1 and 12 h rats during the post-nicotine phase of the experiment (see Figures 4 and 5). Thus, it is likely that similar long-lasting adaptations were manifest in the reward systems of 1 and 12 h rats, but that these adaptations were not apparent in 12 h rats until nicotine was unavailable for self-administration for periods greater than the 12 h between each self-administration session. The precise mechanism that accounts for the above pattern of effects is unclear. It is unlikely, however, that repeated daily exposure to the ICSS procedure contributed to the increased sensitivity in reward systems reported here because control rats, that also had ICSS thresholds assessed twice daily, demonstrated stable and unaltered reward thresholds across days. One possible mechanism that may account for the present observations is that nAChRs located in brain reward systems become desensitized and subsequently upregulated during repeated 1 and 12 h daily nicotine self-administration sessions. Indeed, it was recently shown that nicotine self-administration up-regulated nAChRs throughout the rat brain (Parker *et al*, 2004). Thus, when nicotine is unavailable for self-administration during extended periods of time, such as the 23 h between each self-administration session in 1 h rats or during the post-nicotine phase of the experiment in 1 and 12 h rats, nAChRs may recover from nicotine-induced desensitization and enter a state of hyperactivity, inducing hyperexcitability of brain reward systems. By a similar line of reasoning, nAChRs located in brain circuits that regulate other behavioral aspects of

self-administered nicotine, such as its anxiogenic effects (Irvine *et al*, 2001), may also become hyperactive after cessation of nicotine intake, contributing to the well-characterized aversive syndrome observed in human smokers during extended periods of nicotine abstinence.

The hypersensitivity of brain reward systems that emerged during the post-nicotine phase of the experiment closely resembles the reward hypersensitivity that emerges in food-restricted rats undergoing a period of negative energy balance (Blundell and Herberg, 1968; Carr and Wolinsky, 1993; Carr, 1996; Lin *et al*, 2002). This reward hypersensitivity during food deprivation is hypothesized to increase the appetitive value of food and thereby drive food intake (Herberg and Blundell, 1967; Fulton *et al*, 2000). Thus, it is possible that the reward hypersensitivity during nicotine deprivation may be analogous, albeit maladaptive, to that observed in food-deprived rats, increasing the appetitive value of nicotine and perpetuating the nicotine habit. A number of other possible consequences of the long-lasting stimulatory action of nicotine on reward pathways may also be postulated. Nicotine-induced reward hypersensitivity may potentiate the effects of other drug reinforcers, and thereby account for the high incidence of abuse such as alcohol (Hughes, 1994) or caffeine (Brown and Benowitz, 1989; Shoaib *et al*, 1999). In addition, nicotine-induced reward hypersensitivity may potentiate the effects of nondrug, natural reinforcers. Such an action could potentially contribute to the increased food intake, and subsequent weight gain often observed in abstinent human smokers. In addition, nicotine-induced potentiation of nondrug reinforcers may help explain recent observations that nicotine facilitates reward-related learning (Olausson *et al*, 2003), increases responding for a conditioned reinforcer previously paired with water reward (Olausson *et al*, 2004a,b), potentiates responding for a natural (visual) reinforcer (Donny *et al*, 2003), and accounts for the long-lasting motivational effects of nicotine-associated conditioned reinforcers (Cohen *et al*, 2005). The reward hypersensitivity that persists after nicotine intake has ceased may also serve as a powerful interoceptive discriminative stimulus. Indeed, relapse to the smoking habit can occur after many years of abstinence, suggesting that enduring nicotine-induced adaptations may persist in the brains of smokers. Thus, it is possible that reward hypersensitivity in abstinent human smokers acts as a persistent 'memory' in reward pathways of the effects of nicotine, which provokes relapse even after long periods of smoking abstinence.

In summary, nicotine self-administration increased the sensitivity of brain reward systems, and this action of nicotine persisted for at least 36 days after nicotine intake had ceased. Acute nicotine consumption stimulates reward pathways in a manner similar to other drugs of abuse, a factor likely essential in establishing and maintaining the tobacco-smoking habit in humans. However, nicotine may imprint an indelible 'memory' in brain reward pathways by resetting their sensitivity to a new increased level, a property that so far appears unique to nicotine among drugs of abuse. Overall, these data represent a fundamental departure from previous conceptualizations of the long-term actions of nicotine on brain reward systems, and

provide a novel framework for understanding the motivational drives that contribute to tobacco addiction in humans.

## ACKNOWLEDGEMENTS

This work was supported by the National Association for Research on Schizophrenia and Depression (NARSAD; PJK), and the Tobacco Related Disease Research Program from the State of California (12RT-0231; AM). We thank Drs Stephanie Caille and Marisa Roberto for helpful comments on an earlier version of the manuscript. We are especially grateful to Mr Michael Arends for insightful comments and discussions, and for help with manuscript preparation. This is publication number 16692-NP from The Scripps Research Institute.

## REFERENCES

- Ahmed SH, Kenny PJ, Koob GF, Markou A (2002). Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. *Nat Neurosci* 5: 625–626.
- Ahmed SH, Koob GF (1998). Transition from moderate to excessive drug intake: change in hedonic set point. *Science* 282: 298–300.
- Ahmed SH, Walker JR, Koob GF (2000). Persistent increase in the motivation to take heroin in rats with a history of drug escalation. *Neuropsychopharmacology* 22: 413–421.
- Annau Z, Heffner R, Koob GF (1974). Electrical self-stimulation of single and multiple loci: long term observations. *Physiol Behav* 13: 281–290.
- Bauco P, Wise RA (1997). Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. *J Pharmacol Exp Ther* 283: 1160–1167.
- Bespalov A, Dumpis M, Piotrovsky L, Zvartau E (1994). Excitatory amino acid receptor antagonist kynurenic acid attenuates rewarding potential of morphine. *Eur J Pharmacol* 264: 233–239.
- Blundell JE, Herberg LJ (1968). Relative effects of nutritional deficit and deprivation period on rate of electrical self-stimulation of lateral hypothalamus. *Nature* 219: 627–628.
- Brown CR, Benowitz NL (1989). Caffeine and cigarette smoking: behavioral, cardiovascular, and metabolic interactions. *Pharmacol Biochem Behav* 34: 565–570.
- Caine B, Koob GF (1993). Intravenous drug self-administration techniques in animals. In: Sahgal A (ed). *Behavioural Neuroscience: A Practical Approach*. IRL Press: Oxford. pp 117–143.
- Carr KD (1996). Feeding, drug abuse, and the sensitization of reward by metabolic need. *Neurochem Res* 21: 1455–1467.
- Carr KD, Wolinsky TD (1993). Chronic food restriction and weight loss produce opioid facilitation of perifornical hypothalamic self-stimulation. *Brain Res* 607: 141–148.
- Cohen C, Perrault G, Griebel G, Soubrie P (2005). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). *Neuropsychopharmacology* 30: 145–155.
- Damaj MI, Welch SP, Martin BR (1995). *In vivo* pharmacological effects of dihydro-beta-erythroidine, a nicotinic antagonist, in mice. *Psychopharmacology (Berl)* 117: 67–73.
- Donny EC, Chaudhri N, Caggiula AR, Evans-Martin FF, Booth S, Gharib MA *et al* (2003). Operant responding for a visual reinforcer in rats is enhanced by noncontingent nicotine: implications for nicotine self-administration and reinforcement. *Psychopharmacology (Berl)* 169: 68–76.
- Epping-Jordan MP, Picciotto MR, Changeux JP, Pich EM (1999). Assessment of nicotinic acetylcholine receptor subunit con-



- tributions to nicotine self-administration in mutant mice. *Psychopharmacology (Berl)* 147: 25–26.
- Epping-Jordan MP, Watkins SS, Koob GF, Markou A (1998). Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* 393: 76–79.
- Fulton S, Woodside B, Shizgal P (2000). Modulation of brain reward circuitry by leptin. *Science* 287: 125–128.
- Harrison AA, Gasparini F, Markou A (2002). Nicotine potentiation of brain stimulation reward reversed by DH $\beta$ E and SCH 23390, but not by eticlopride, LY 314582 or MPEP in rats. *Psychopharmacology (Berl)* 160: 56–66.
- Harvey SC, Luetje CW (1996). Determinants of competitive antagonist sensitivity on neuronal nicotinic receptor beta subunits. *J Neurosci* 16: 3798–3806.
- Harvey SC, Maddox FN, Luetje CW (1996). Multiple determinants of dihydro-beta-erythroidine sensitivity on rat neuronal nicotinic receptor alpha subunits. *J Neurochem* 67: 1953–1959.
- Herberg LJ, Blundell JE (1967). Lateral hypothalamus: hoarding behavior elicited by electrical stimulation. *Science* 155: 349–350.
- Hughes JR (1994). Smoking and alcoholism. In: Cos JL, Hatsukami DK (eds). *Behavioral Approaches to Addiction*. Cahners Healthcare Communications: Belle Mead, New Jersey.
- Irvine EE, Bagnalasta M, Marcon C, Motta C, Tessari M, File SE *et al* (2001). Nicotine self-administration and withdrawal: modulation of anxiety in the social interaction test in rats. *Psychopharmacology (Berl)* 153: 315–320.
- Kenny PJ, Polis I, Koob GF, Markou A (2003). Low dose cocaine self-administration transiently increases but high dose cocaine persistently decreases brain reward function in rats. *Eur J Neurosci* 17: 191–195.
- Kornetsky C, Esposito RU (1979). Euphorogenic drugs: effects on the reward pathways of the brain. *Fed Proc* 38: 2473–2476.
- Kornetsky C, Esposito RU, McLean S, Jacobson JO (1979). Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. *Arch Gen Psychiatry* 36: 289–292.
- Lin D, Bruijnzeel AW, Schmidt P, Markou A (2002). Exposure to chronic mild stress alters thresholds for lateral hypothalamic stimulation reward and subsequent responsiveness to amphetamine. *Neuroscience* 114: 925–933.
- Mansvelder HD, McGehee DS (2000). Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron* 27: 349–357.
- Markou A, Koob GF (1991). Postcocaine anhedonia. An animal model of cocaine withdrawal. *Neuropsychopharmacology* 4: 17–26.
- Markou A, Koob GF (1992). Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol Behav* 51: 111–119.
- Olausson P, Jentsch JD, Taylor JR (2003). Repeated nicotine exposure enhances reward-related learning in the rat. *Neuropsychopharmacology* 28: 1264–1271.
- Olausson P, Jentsch JD, Taylor JR (2004a). Nicotine enhances responding with conditioned reinforcement. *Psychopharmacology (Berl)* 171: 173–178.
- Olausson P, Jentsch JD, Taylor JR (2004b). Repeated nicotine exposure enhances responding with conditioned reinforcement. *Psychopharmacology (Berl)* 173: 98–104.
- Olds J (1958). Satiation effects in self-stimulation of the brain. *J Comp Physiol Psychol* 51: 675–678.
- Olds J, Milner PM (1954). Positive reinforcement produced by electrical stimulation of the septal area and other regions of rat brain. *J Comp Physiol Psychol* 47: 419–427.
- Parker SL, Fu Y, McAllen K, Luo J, McIntosh JM, Lindstrom JM *et al* (2004). Up-regulation of brain nicotinic acetylcholine receptors in the rat during long-term self-administration of nicotine: disproportionate increase of the alpha6 subunit. *Mol Pharmacol* 65: 611–622.
- Paterson NE, Markou A (2004). Prolonged nicotine dependence associated with extended access to nicotine self-administration in rats. *Psychopharmacology (Berl)* 173: 64–72.
- Pellegrino LJ, Pellegrino AS, Cushman AJ (1979). *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press.
- Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM *et al* (1998). Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. *Nature* 391: 173–177.
- Picciotto MR, Zoli M, Zachariou V, Changeux JP (1997). Contribution of nicotinic acetylcholine receptors containing the beta 2-subunit to the behavioural effects of nicotine. *Biochem Soc Trans* 25: 824–829.
- Rice ME, Cragg SJ (2004). Nicotine amplifies reward-related dopamine signals in striatum. *Nat Neurosci* 7: 583–584.
- Shoaib M, Swanner LS, Yasar S, Goldberg SR (1999). Chronic caffeine exposure potentiates nicotine self-administration in rats. *Psychopharmacology (Berl)* 142: 327–333.
- Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C *et al* (2004). Nicotine activation of alpha4\* receptors: sufficient for reward, tolerance, and sensitization. *Science* 306: 1029–1032.
- Watkins SS, Stinus L, Koob GF, Markou A (2000). Reward and somatic changes during precipitated nicotine withdrawal in rats: centrally and peripherally mediated effects. *J Pharmacol Exp Ther* 292: 1053–1064.
- Wise RA (1996). Addictive drugs and brain stimulation reward. *Annu Rev Neurosci* 19: 319–340.
- Wise RA, Yokel RA, Hansson PA, Gerber GJ (1977). Concurrent intracranial self-stimulation and amphetamine self-administration in rats. *Pharmacol Biochem Behav* 7: 459–461.