Phasic D1 and tonic D2 dopamine receptor signaling double dissociate the motivational effects of acute nicotine and chronic nicotine withdrawal

Taryn E. Grieder[a,1], Olivier George[b], Huibing Tan[c], Susan R. George[d], Bernard Le Foll[a,d], Steven R. Laviolette[c], and Derek van der Kooy[a]

[a]Institute of Medical Science, University of Toronto, Toronto, ON, Canada M5S 3E1; [b]Committee on the Neurobiology of Addictive Disorders, The Scripps Research Institute, La Jolla, CA 92037; [c]Schulich School of Medicine, University of Western Ontario, London, ON, Canada N5Y 5T8; and [d]Centre for Addiction and Mental Health, Toronto, ON, Canada M5S 2S1

Edited by Leslie Lars Iversen, University of Oxford, Oxford, United Kingdom, and approved December 20, 2011 (received for review September 2, 2011)

Nicotine, the main psychoactive ingredient of tobacco smoke, induces negative motivational symptoms during withdrawal that contribute to relapse in dependent individuals. The neurobiological mechanisms underlying how the brain signals nicotine withdrawal remain poorly understood. Using electrophysiological, genetic, pharmacological, and behavioral methods, we demonstrate that tonic but not phasic activity is reduced during nicotine withdrawal in ventral tegmental area dopamine (DA) neurons, and that this pattern of signaling acts through DA D2 and adenosine A2A, but not DA D1, receptors. Selective blockade of phasic DA activity prevents the expression of conditioned place aversions to a single injection of nicotine in nondependent mice, but not to withdrawal from chronic nicotine in dependent mice, suggesting a shift from phasic to tonic dopaminergic mediation of the conditioned motivational response in nicotine dependent and withdrawn animals. Either increasing or decreasing activity at D2 or A2A receptors prevents the aversive motivational response to withdrawal from chronic nicotine, but not to acute nicotine. Modification of D1 receptor activity prevents the aversive response to acute nicotine, but not to nicotine withdrawal. This double dissociation demonstrates that the specific pattern of tonic DA activity at D2 receptors is a key mechanism in signaling the motivational effects experienced during nicotine withdrawal, and may represent a unique target for therapeutic treatments for nicotine addiction.

negative reinforcement | place conditioning | burst firing | population activity | osmotic minipumps

Tobacco addiction is the leading avoidable cause of disease and premature death in North America (1). Of more than 3,000 chemicals present in tobacco smoke, nicotine is the main psychoactive ingredient responsible for tobacco addiction (2). Withdrawal from chronic nicotine is hypothesized to represent a powerful source of negative reinforcement that drives relapse and compulsive tobacco use (3); therefore, understanding the neurobiological substrates mediating the motivational properties of nicotine withdrawal is an important step in the development of new treatments for nicotine addiction. Current hypotheses suggest that nicotine withdrawal leads to a decrease in dopamine (DA) signaling in the brain (4) and that DA neurons exhibit two activity states, phasic and tonic, that mediate separate aspects of behavior (5–7).

Nicotine acutely produces both aversive and positive motivational effects (8, 9) by activating the mesolimbic DA system (7, 10) as well as non-DAergic neural substrates (11, 12). DA neurons exhibit burst- and population-firing activity that leads to phasic and tonic DA release, respectively (5–7). Burst-firing produces a fast and large phasic DA release that mainly activates postsynaptic DA D1 receptors (D1Rs), and population-firing produces a slower tonic DA release that mainly activates the higher affinity (13) DA D2 receptors (D2Rs) (5, 6). The phasic and tonic activities of DA neurons are thought to mediate different aspects of goal-directed behavior; phasic activity facilitates cue-reward association and acquisition of incentive salience, whereas tonic activity is involved in response inhibition and behavioral flexibility (5, 6, 14). Consistent with its motivational properties, a single systemic nicotine injection increases phasic activity in the ventral tegmental area (VTA) (15) and the release of DA in the ventral striatum (16, 17), and chronic exposure to nicotine decreases tonic, but not phasic, DA activity in the VTA (18). However, the role of tonic activity and the D1R vs. phasic activity and the D2R in signaling the motivational effects of acute nicotine and withdrawal from chronic nicotine is unknown. Here we tested the effect of withdrawal from chronic nicotine on tonic and phasic VTA DA activity and whether the specific pattern of signaling through D1Rs and D2Rs mediates the conditioned motivational responses to nicotine withdrawal and acute nicotine.

Results

Activation or Blockade of DA Receptors Prevents the Expression of Chronic Nicotine Withdrawal Aversions. Pharmacological blockade of DA activity at receptors attenuates the expression of food (19) and drug motivation (20, 21) in place-conditioning paradigms. Interestingly, pharmacological activation of DA receptors (DARs) also prevents food motivation (19) and conditioned place aversions (CPAs) to morphine withdrawal (21). We hypothesized that a specific pattern of signaling at DARs could mediate nicotine withdrawal, and thus that either pharmacologically increasing or decreasing activity at DARs would prevent CPAs to nicotine withdrawal. Mice given chronic nicotine (7 mg·kg·d) were subjected to place-conditioning during spontaneous withdrawal (20) after pretreatment with vehicle, the DAR agonist apomorphine (2.5 mg/kg), or the DAR antagonist α-flupenthixol (0.8 mg/kg). A one-way ANOVA showed a significant effect of pharmacological pretreatment ($F_{2,42} = 17.1$, $P < 0.05$) (Fig. 1*A*) on the motivational response to nicotine withdrawal. Dependent and withdrawn mice pretreated with vehicle ($n = 15$) showed a significant aversion to a withdrawal-paired environment ($P < 0.05$) that was blocked with apomorphine ($n = 15$; $P > 0.05$) or α-flupenthixol ($n = 15$; $P > 0.05$) pretreatment. Each drug pretreatment had no motivational effects on its own (Fig. S1). Similar to previous results in chronic opiate withdrawn rats (19), these results suggest that disruption of the specific pattern of DA signaling by either increasing or

Author contributions: T.E.G., O.G., S.R.G., B.L.F., and D.v.d.K. designed research; T.E.G., H.T., and S.R.L. performed research; T.E.G. and O.G. analyzed data; and T.E.G., O.G., and D.v.d.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

See Commentary on page 2697.

[1]To whom correspondence should be addressed: E-mail: taryn.grieder@utoronto.ca.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1114422109/-/DCSupplemental.

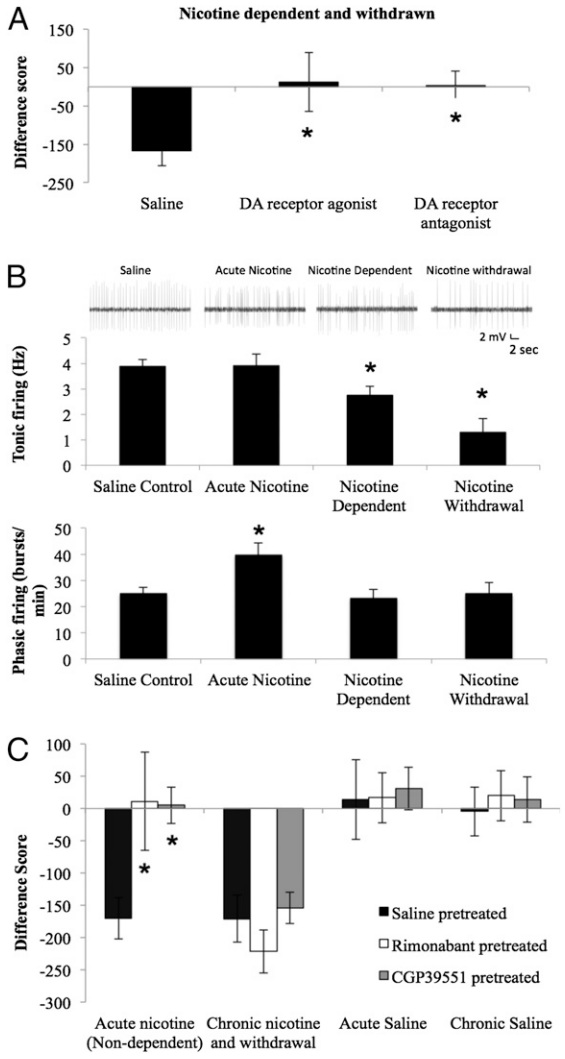

Fig. 1. Phasic DA activity mediates aversions to acute nicotine but the specific pattern of tonic DA activity mediates aversions to withdrawal from chronic nicotine. (*A*) Both increasing and decreasing DAR activity prevents the expression of withdrawal aversions. Nicotine-dependent and -withdrawn mice pretreated with vehicle showed an aversion to a withdrawal-paired environment that was blocked (*$P < 0.05$) after pretreatment with DAR agonist apomorphine or DAR antagonist α-flupenthixol. (*B*) (*Top*) Representative electrophysiological recordings from VTA DA neurons in rats treated with saline vehicle, acute nicotine, chronic nicotine (nicotine-dependent), and chronic nicotine and spontaneous withdrawal. (*Middle*) Nicotine-dependent rats exhibited a decrease in tonic VTA DA activity compared with saline control and acute nicotine-treated rats that was further significantly decreased in rats undergoing withdrawal from chronic nicotine (*$P < 0.05$ in comparison with all other groups). (*Bottom*) Only acute nicotine increased phasic activity in VTA DA neurons. Chronic nicotine exposure and withdrawal did not alter phasic activity. (*C*) Selectively blocking phasic DA activity with rimonabant or CGP39551 prevented aversions to acute nicotine but not to nicotine withdrawal. Data represent mean ± SEM (*$P < 0.05$).

decreasing activity at DARs prevents the expression of nicotine withdrawal aversions in dependent mice.

Tonic but Not Phasic DA Activity in the VTA Is Altered in Nicotine-Dependent and Withdrawn Rats. We next directly investigated the specific patterns of DA neuron firing that mediate the motivational response to nicotine withdrawal. Using defined criteria to measure phasic bursting activity and tonic population activity of DA neurons (18, 22), we measured tonic and phasic VTA DA activity with in vivo extracellular single-unit recordings in saline control, previously drug-naive given acute nicotine (1.5 mg/kg), nicotine dependent (3.14 mg·kg·d), and nicotine-dependent and spontaneously withdrawn rats (20, 23). Analysis of tonic DA neuron activity with one-way ANOVA revealed a significant effect of drug treatment ($F_{3,35} = 9.7$, $P < 0.05$) (Fig. 1*B*). Saline-treated ($n = 11$) and acute nicotine-treated ($n = 11$) rats showed no difference in tonic DA neuron activity ($P > 0.05$). However, similar to previous studies (18, 24), nicotine-dependent rats receiving chronic nicotine ($n = 11$) showed a significant decrease in tonic DA activity in comparison with both saline controls and acute nicotine-treated groups ($P < 0.05$). Most interesting, rats experiencing withdrawal from chronic nicotine ($n = 6$) showed a further decrease in tonic DA activity compared with nicotine-dependent rats that were not in withdrawal ($P < 0.05$). This result is consistent with the hypothesis that dependent human smokers have decreased DA activity during withdrawal (4), and suggests that the aversive motivational state of spontaneous nicotine withdrawal is signaled by a further patterned decrease in tonic DA activity than that observed during the nicotine-dependent state. Analysis of phasic VTA DA activity with one-way ANOVA revealed a significant effect of drug treatment ($F_{3,35} = 5.0$, $P < 0.05$) (Fig. 1*B*). Acute nicotine increased phasic DA firing rates ($P < 0.05$), in comparison with nondependent, dependent, and dependent and withdrawn groups (all $P > 0.05$), suggesting that the specific pattern of phasic activity may mediate the motivational response to acute nicotine.

Blockade of Phasic DA Activity Prevents Aversions to Acute Nicotine, but Not to Withdrawal from Chronic Nicotine. To test if phasic DA activity directly mediates the aversive response to acute nicotine, but not to chronic nicotine withdrawal, we examined the effect of blocking phasic DA activity on CPAs for acute nicotine and withdrawal from chronic nicotine using the cannabinoid receptor-1 inverse agonist rimonabant (3.0 mg/kg) and the NMDA receptor antagonist CGP39551 (2.5 mg/kg). Previous studies suggested that rimonabant blocks phasic DA release without affecting baseline DA transients (25, 26); however, these were performed in vitro (25) or measured the absolute amount of DA release using voltammetry (26) or microdialysis (27). We thus performed in vivo electrophysiological recordings of VTA DA neurons in drug-naive rats given rimonabant ($n = 10$) to test the hypothesis that the drug selectively decreases phasic but not tonic baseline DA firing. Rimonabant significantly decreased phasic DA activity ($t_9 = 2.715$, $P < 0.05$) but not tonic DA activity ($t_9 = 0.2018$, $P > 0.05$) (Fig. S2). CGP39551 is another pharmacological tool that selectively disrupts phasic DA activity without affecting tonic activity and blocks nicotine-induced VTA DA bursting (28). A two-way ANOVA showed a significant interaction of pharmacological pretreatment and nicotine history ($F_{6,110} = 4.291$, $P < 0.05$) (Fig. 1*C*). Nondependent mice given acute nicotine after saline pretreatment ($n = 9$) showed a CPA to a nicotine-paired environment ($P < 0.05$) that was blocked with rimonabant ($n = 9$; $P > 0.05$) or CGP39551 ($n = 13$; $P > 0.05$) pretreatment. In contrast, nicotine-dependent and withdrawn mice given saline ($n = 11$) showed a CPA to the withdrawal-paired environment ($P < 0.05$) that was not blocked by rimonabant ($n = 13$; $P < 0.05$) or CGP39551 ($n = 12$, $P < 0.05$). Mice given chronic or acute saline and saline ($n = 14$), rimonabant ($n = 7$), or CGP39551 ($n = 6$) showed no motivational response to the drugs ($P > 0.05$). These results suggest that phasic DA activity is required for the motivational response to acute nicotine, but not to withdrawal from chronic nicotine.

Genetic Deletion of D2Rs vs. D1Rs Double Dissociate Chronic vs. Acute Nicotine Motivation. Phasic and tonic DA signaling act through D1Rs and D2Rs, respectively (5, 6, 14), and we demonstrated here

that acute nicotine modifies phasic DA activity, but withdrawal from chronic nicotine modifies tonic DA activity. We thus hypothesized that genetic deletion of the D2R would prevent aversions to nicotine withdrawal in dependent mice, but D1R deletion would prevent acute nicotine aversions in nondependent mice. D1R and D2R KO mice and their WT littermates were place-conditioned after receiving acute nicotine (1.75 mg/kg) or during spontaneous withdrawal from chronic nicotine (7 mg·kg·d). One-way ANOVA revealed a significant group effect ($F_{2,24} = 3.43$, $P < 0.05$) (Fig. 2*A*) in dependent and withdrawn mice. WT mice in withdrawal from chronic nicotine ($n = 11$) showed a CPA to a withdrawal-paired environment ($P < 0.05$); aversions that were shown as well by D1R KO mice ($n = 9$) but not by D2R KO mice ($n = 7$; $P > 0.05$). For acute nicotine-treated mice, one-way ANOVA revealed a significant group effect ($F_{2,27} = 8.27$, $P < 0.05$) (Fig. 2*B*). D2R KO ($n = 7$) and previously drug-naive WT mice ($n = 14$) given acute nicotine showed a significant CPA to a nicotine-paired environment ($P < 0.05$) that was blocked in D1R KO mice ($n = 9$; $P > 0.05$). Taken together, these results doubly dissociate the role of D1Rs and D2Rs in nicotine motivation; D2Rs (but not D1Rs) are required for the aversive motivational response to withdrawal in dependent mice, and D1Rs (but not D2Rs) are necessary for acute nicotine aversions in nondependent mice.

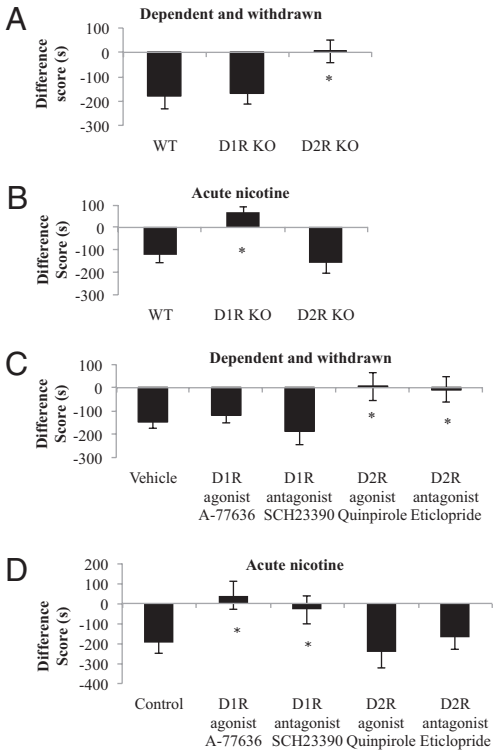

Fig. 2. D1Rs are required for aversions to acute nicotine but the specific pattern of D2R activity is required for aversions to nicotine withdrawal. (*A*) The aversive response to withdrawal from chronic nicotine is observed in D1R but not D2R KOs. (*B*) The aversive response to acute nicotine is blocked in D1R KOs but not in D2R KOs. (*C*) Nicotine-dependent and -withdrawn mice pretreated with vehicle show a CPA to a withdrawal-paired environment that is blocked with D2R antagonist eticlopride and D2R agonist quinpirole pretreatment, but not with D1R antagonist SCH23390 or D1R agonist A-77636 pretreatment. (*D*) Nondependent mice given an injection of nicotine and pretreated with vehicle showed a CPA to a nicotine-paired environment that was blocked with A-77636 and SCH23390 pretreatment, but not with quinpirole or eticlopride pretreatment. Data represent mean ± SEM (*$P < 0.05$).

Pharmacological Manipulation of D2Rs but Not D1Rs Block Withdrawal Aversions in Nicotine-Dependent Mice. Modification of the specific pattern of activity at DARs, and D2R but not D1R deletion, blocked the aversive response to withdrawal from chronic nicotine. We thus hypothesized that either increasing or decreasing activity at D2Rs but not D1Rs would block conditioned withdrawal aversions in dependent mice. We examined the effect of the D1R agonist A-77636 (1.0 mg/kg), the D1R antagonist SCH23390 (0.01 mg/kg), the D2R agonist quinpirole (0.05 mg/kg), and the D2R antagonist eticlopride (1.0 mg/kg) on CPAs to withdrawal. A one-way ANOVA revealed a significant effect of pharmacological pretreatment ($F_{4,60} = 4.11$ $P < 0.05$) (Fig. 2*C*). Nicotine-dependent and -withdrawn mice that received vehicle ($n = 31$) before conditioning showed an aversion to a withdrawal-paired environment ($P < 0.05$) that was blocked in mice that received quinpirole ($n = 7$; $P > 0.05$) and eticlopride ($n = 7$; $P > 0.05$), but not in mice that received A-77636 ($n = 12$; $P < 0.05$) or SCH23390 ($n = 8$; $P < 0.05$). No motivational response to any of the drugs on their own was observed (Fig. S3). Groups of mice tested with a higher dose of the D1R agonist A-77636 (10.0 mg/kg) showed a nonspecific block of learning (Fig. S4). These results suggest that either increasing or decreasing D2R activity blocks the specific pattern of signaling that mediates the aversive motivational response to chronic nicotine withdrawal, and that activity at D1Rs is not required for the experience of nicotine withdrawal aversions.

Pharmacological Manipulations of D1Rs but Not D2Rs Block Aversions to Acute Nicotine in Nondependent Mice. We next tested the hypothesis that either increasing or decreasing activity at D1Rs but not D2Rs would prevent the aversive motivational response to acute nicotine by examining the effect of A-77636, SCH23390, quinpirole, and eticlopride on CPAs to a nicotine-paired environment in nondependent mice. There was a significant effect of pharmacological pretreatment on acute nicotine aversions ($F_{4,44} = 4.99$ $P < 0.05$) (Fig. 2*D*) that occurred exactly opposite to dependent and withdrawn mice. Nondependent mice given acute aversive nicotine and pretreated with vehicle ($n = 17$) showed a CPA to a nicotine-paired environment ($P < 0.05$) that was blocked in mice pretreated with the D1R agonist A-77636 ($n = 8$; $P > 0.05$) and the D1R antagonist SCH23390 ($n = 10$; $P > 0.05$), but not with the D2R agonist quinpirole ($n = 7$; $P < 0.05$) or the D2R antagonist eticlopride ($n = 7$; $P < 0.05$). These results demonstrate that either increasing or decreasing activity at D1Rs blocks aversions to acute nicotine, and suggest that D2R activation is not necessary for the aversive response to acute nicotine.

Pharmacological and Genetic Modifications of Adenosine A2A Receptors Specifically Block Chronic Nicotine Withdrawal Aversions. In the striatum, adenosine A2A receptors (A2ARs) and D2Rs are colocalized (29) and form A2AR-D2R heteromers (30). The A2AR and D2R interact antagonistically, such that agonism of A2ARs decreases signaling at D2Rs (31) and antagonism of A2ARs increases signaling at D2Rs (30). If the specific pattern of activity at D2Rs is a key factor in mediating aversions to nicotine withdrawal, and colocalized A2ARs and D2Rs act antagonistically in the striatum (29, 30), then genetic and pharmacological manipulation of A2ARs should also affect nicotine-withdrawal aversions in dependent animals. We examined the effect of A2AR manipulation on the conditioned aversive responses to acute nicotine and withdrawal from chronic nicotine in A2AR KO mice and WT mice pretreated with the A2AR agonist CGS21680 (0.1 mg/kg) or the A2AR antagonist SCH58261 (0.5 mg/kg). One-way ANOVA revealed a significant effect of A2A receptor manipulation in nicotine-dependent and -withdrawn mice ($F_{3,51} = 6.2$ $P < 0.05$) (Fig. 3*A*) but not in nondependent mice given acute nicotine ($F_{2,24} = 0.06$ $P > 0.05$) (Fig. 3*B*). Dependent and

withdrawn WT mice that received vehicle ($n = 23$) showed a CPA to a withdrawal-paired environment ($P < 0.05$) that was blocked in A2AR KO mice ($n = 14$; $P > 0.05$) and in WT mice that received CGS21680 ($n = 7$; $P > 0.05$) or SCH58261 ($n = 11$; $P > 0.05$). Previously drug-naive mice given acute nicotine and pretreated with vehicle ($n = 13$) showed a CPA to a nicotine-paired environment that was not blocked in mice pretreated with CGS21680 ($n = 7$; $P > 0.05$) or SCH58261 ($n = 7$; $P > 0.05$). No motivational response to the drugs on their own was observed (Fig. S5). These results suggest that either increasing or decreasing activity at A2ARs blocks aversions to withdrawal from chronic nicotine but not the aversive response to acute nicotine, possibly via modification of D2R activity.

Discussion

Withdrawal from nicotine has been hypothesized to represent a powerful source of negative reinforcement (20, 23) that drives relapse and compulsive tobacco use (2, 3). Therefore, understanding the neurobiological substrates mediating the motivational properties of withdrawal from chronic nicotine is an important step in the development of new treatments for nicotine addiction. Previous reports have suggested that a neurobiological switch occurs during the transition from a drug-naive to a drug-dependent motivational state (32). The transition from acute nicotine use to nicotine dependence has been hypothesized to result from neuroadaptative changes that produce the powerful withdrawal syndrome and negative emotional state observed upon cessation of nicotine use (3). The present results demonstrate that a shift in VTA DA signaling from phasic to tonic, and of receptor mediation from D1 to D2, occurs upon dependence and withdrawal from nicotine, and doubly dissociates the role of D1Rs vs. D2Rs in nicotine motivation. We suggest that phasic DA activity at D1Rs mediates acute nicotine aversions, whereas tonic DA activity at D2Rs (and indirectly, A2ARs) mediates aversions to withdrawal from chronic nicotine.

Rodents experiencing spontaneous withdrawal from chronic nicotine show a CPA to a withdrawal-paired environment in place-conditioning paradigms (20, 33). Similarly, previously drug-naive mice given a single aversive dose of nicotine will show a CPA to the nicotine-paired environment (20). The important difference in these two effects is the exposure to nicotine: acute exposure in nondependent animals versus chronic exposure and withdrawal in dependent animals. The present results demonstrate that modifying activity at D2Rs prevented the expression of nicotine-withdrawal aversions in dependent animals. A previous study suggested that both increasing or decreasing DA signaling at DARs blocked the expression of CPAs to withdrawal from chronic morphine (21). Our present work suggests that a similar phenomenon occurs during nicotine withdrawal in dependent animals, such that treatment with a broad-spectrum DAR agonist or antagonist, or a specific D2R agonist or antagonist, prevented the expression of nicotine-withdrawal aversions in dependent mice. Furthermore, genetic deletion of the D2R (but not the D1R) prevented nicotine-withdrawal aversions. In nondependent animals exposed to acute nicotine, exactly the opposite phenomenon occurred: Both D1R-specific agonism or antagonism, as well as D1R deletion, selectively prevented aversions to acute nicotine in nondependent mice, without affecting aversions to withdrawal in nicotine-dependent mice. These results doubly dissociate the role of the D1R vs. D2R in nicotine motivation, such that the motivational response to withdrawal in dependent mice is D2R-mediated and acute nicotine motivation is D1R-mediated. These results are in line with previous studies showing that drug-dependent human subjects have marked decreases in D2R availability (34) and presumably in DA release (35), which is consistent with the hypothesis that a pattern of DA activity signals nicotine motivation and the present results showing that nicotine-dependent and -withdrawn mice have a decrease in tonic activity of VTA DA neurons. Furthermore, animal studies have shown that D1R antagonism blocks nicotine motivation in nondependent mice (36). A recent study showed that blockade of D1R but not D2R transmission prevented acquisition of opiate-reward memory in nondependent rats, and D2R but not D1R blockade prevented opiate-reward encoding in dependent and withdrawn rats (37). However, previous studies suggest that both acute nicotine and opiate reward are mediated by the non-DAergic brainstem tegmental pedunculopontine (TPP) nucleus (9, 12, 21), and thus must involve separate cells in the TPP that are thought to mediate burst-firing of VTA DA neurons (5, 14); burst-firing that we show here is involved in the response to acute nicotine. The present data show that only the acute aversive motivational effects of nicotine are mediated by D1Rs, leading to the suggestion that the induction of nicotine dependence switches the neurobiological substrate mediating the aversive motivational effects of nicotine from D1R to D2R-mediated.

We have suggested that a specific pattern of tonic DA activity through D2Rs signals withdrawal from chronic nicotine. The D2R system is important for learning to shift behavior in response to change in motivation (6). It is thus possible that animals have a tonic pattern of DA activity that does not shift with nicotine dependence and withdrawal. D2R KO mice did not demonstrate a CPA to withdrawal, possibly because these mice never experience a change in tonic DA activity that signals withdrawal. However, this block of the motivational response is not simply because of an effect on learning, as both D1R and D2R KO mice can learn a motivational response to nicotine in our paradigm. Indeed, both hyperdopaminergic and hypodopaminergic mice can learn various tasks although their motivation is altered (14), suggesting that DA mediates motivation rather than learning. Our block of nicotine withdrawal aversions with both D2R agonist and antagonist drugs provides further support for the hypothesis that the specific pattern of DA release at D2Rs signals withdrawal, and that any deviation from this pattern, whether an increase or a decrease of DA activity and release, will prevent the aversive motivational response to nicotine withdrawal.

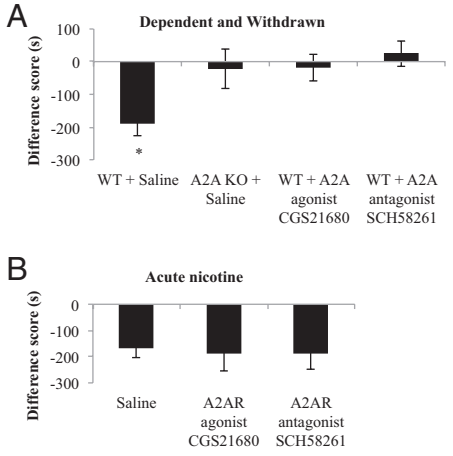

Fig. 3. Manipulations of the adenosine A2AR block the aversive response to withdrawal from chronic nicotine but not to acute nicotine. (*A*) Nicotine-dependent and -withdrawn WT mice pretreated with vehicle showed a CPA to a withdrawal-paired environment that was blocked in A2AR KO mice, mice pretreated with the A2AR agonist CGS21680, and mice pretreated with the A2AR antagonist SCH58261. (*B*) Nondependent mice given an acute injection of nicotine and pretreated with vehicle showed a CPA to a nicotine-paired environment that was not blocked with CGS21680 or SCH58261 pretreatment. Data represent mean ± SEM (*$P < 0.05$).

The present results doubly dissociate the role of phasic and tonic dopaminergic activity in the motivational response to acute nicotine in nondependent mice and to withdrawal in nicotine-dependent mice. Previous studies have shown that tonic DA activity is decreased in nicotine-dependent animals (18) and that precipitated withdrawal from chronic nicotine leads to decreased DA levels (38). Using defined electrophysiological methods to measure VTA DA activity (18, 22), we confirmed and extended these results to dependent animals experiencing spontaneous withdrawal, showing that tonic DA activity is further decreased during withdrawal from chronic nicotine. Acute nicotine increased phasic DA activity in nondependent animals; pharmacologically blocking phasic activity via cannabinoid-1 (33) or NMDA (28) receptor modulation prevented the aversive motivational response to acute nicotine, but not to withdrawal from chronic nicotine. Taken together, these results suggest that nicotine withdrawal is signaled by a pattern of tonic but not phasic DA activity, and that there is a decrease in tonic DA release during withdrawal in dependent animals. Although the amount of DA released via tonic neuronal activity is small in comparison with that via phasic activity, a previous study showed that tonic DA activity is independent of burst-firing and provides sufficient DA to engage behavior (14), thus it is plausible that a tonic DA signal mediates the behavioral response to nicotine withdrawal. A single injection of nicotine leads to the large-scale phasic release of DA (16, 17); therefore, it is possible that nicotine-dependent subjects who are experiencing withdrawal may take nicotine to temporarily modulate DA levels in the brain by increasing release through phasic activation of VTA DA neurons. This hypothesis is similar to Grace's tonic/phasic model of DA system regulation (7). Another possibility suggested by the present results is that acute nicotine floods the DA system in a similar fashion as administration of a broad-spectrum DAR or D2R-specific agonist. These manipulations of DA activity would modify the specific pattern of DA firing that signals withdrawal, and would thus prevent the aversive motivational effects of withdrawal from chronic nicotine.

We suggest that modulation of D2Rs could prevent the motivational effects of nicotine withdrawal; however, directly increasing or decreasing DA activity could potentially produce schizophrenic or Parkinson-like symptoms, respectively. We demonstrate here that both increasing and decreasing activity at adenosine A2ARs blocked nicotine withdrawal aversions in dependent mice but, similar to a previous study (39), had no effect on acute nicotine aversions in nondependent mice. These results suggest that A2AR modulation can prevent the aversive motivational response to nicotine withdrawal, possibly through an indirect disruption of the specific pattern of D2R activity that mediates withdrawal. Furthermore, we hypothesize that tonic and phasic VTA DA activity leads to effects on D1Rs, D2Rs, and A2ARs in the ventral striatum. This idea is supported by a previous study showing that intrastriatal DA antagonism has similar effects to systemic antagonist administration (40). Activation of striatal receptors could in turn feed back to the VTA via direct and indirect pathways (13), and this feedback may be important in the generation of the specific pattern of tonic DA activity that signals nicotine withdrawal aversions.

Taken together, our results suggest that a key mechanism signaling nicotine withdrawal is tonic activity of VTA DA neurons, which may act through D2Rs and indirectly, A2ARs, to signal an aversive motivational state during withdrawal in dependent subjects that contributes to relapse. Pharmacological manipulation of the tonic DA signal prevents the aversive motivational state that is normally experienced during nicotine withdrawal, suggesting that modifying tonic DA activity via manipulation of D2Rs or possibly A2ARs may represent a unique target for therapeutic treatments of nicotine addiction.

Materials and Methods

All animal use procedures were approved by the University of Toronto Animal Care Committee, in accordance with Canadian Council on Animal Care guidelines. Adult male Wistar rats and C57BL/6 mice (Charles River) and D1R, D2R, and A2AR KO mice were housed in a temperature-controlled room with lights on from 7:00 AM to 7:00 PM. Heterozygous D2R breeder mice were received as a gift from D. K. Grandy and M. J. Low (Oregon Health and Science University, Portland, OR), homozygous D1R KO mice from S. George, and heterozygous A2AR mice from M. Schwarszchild and J. Chen (Massachusetts General Hospital, Boston, MA). Crosses were bred at the University of Toronto to obtain homozygous D2R and A2AR KO mice and their WT controls.

Drugs. Nicotine hydrogen tartrate salt (Sigma-Aldrich) was dissolved in saline at pH 7.0 ± 0.3 and administered via osmotic minipumps (chronic nicotine, 7 mg·kg·d) or subcutaneous injection (acute nicotine, 1.75 mg/kg). The DAR agonist apomorphine (2.5 mg/kg), DAR antagonist α-flupenthixol (0.8 mg/kg), D1R antagonist SCH23390 (0.01 mg/kg), D1R agonist A-77636 (1.0 or 10.0 mg/kg), D2R agonist quinpirole (0.05 mg/kg), D2R antagonist eticlopride (1.0 mg/kg), adenosine A2AR antagonist SCH58261 (0.5 mg/kg), and A2AR agonist CGS21680 (0.1 mg/kg) were purchased from Sigma-Aldrich, dissolved in PBS, and administered intraperitoneally 0, 60, 10, 0, 15, 20, 30, and 20 min before conditioning, respectively. The NMDA-R antagonist CGP39551 (2.5 mg/kg) was purchased from Tocris, dissolved in PBS, and administered intraperitoneally immediately before conditioning. Rimonabant (3.0 mg/kg; National Institute on Drug Abuse) was suspended in 0.3% Tween80 in saline and administered intraperitoneally 45 min before conditioning. All doses of drugs are expressed as milligram of free base per kilogram of body weight. Doses and time of injections were selected based on previous studies (19, 20, 28, 41–44).

Electrophysiology. Rats were subcutaneously implanted with osmotic minipumps (model 2001; Alzet) delivering either saline (nondependent) or nicotine (nicotine dependent; 3.14 mg·kg·d) for 7–10 d. Nicotine-dependent and -withdrawn rats had their minipump removed 16–24 h before electrophysiological recordings, a time that corresponds to peak motivational withdrawal (20, 23). VTA (AP: −5.3 mm; ML: ±0.5–0.8 mm; DV: 7–8.5 mm) DA neurons were identified according to well-established electrophysiological features (18, 22), and electrophysiological recordings were performed as previously described (18). Phasic bursting activity of DA neurons was defined as the occurrence of two or more consecutive spikes with an interspike interval lower than 80 ms and terminating with an interspike interval greater than 160 ms. Tonic activity was defined as the baseline firing rate (2–5 Hz) of the DA neuron (18, 22) and did not include a measure of the number of neurons firing.

Place Conditioning. The place-conditioning apparatus was obtained from Med Associates (SOF-700RA-25 Two Chamber Place Preference Apparatus). One environment was black with a metal rod floor and the other was white with a wire mesh floor. An intermediate gray area housed a removable partition. Each cage was cleaned between animals and each group was fully counterbalanced. Mice were implanted with osmotic minipumps (model 1002; Alzet) or given acute nicotine and pretreated intraperitoneally with saline, apomorphine, α-flupenthixol, rimonabant, SCH23390, A-77636, quinpirole, eticlopride, SCH58261, or CGS21680 and conditioned according to modified place-conditioning procedures, as described previously (20) and in *SI Materials and Methods*.

Statistical Analysis. Results were analyzed using a one- or two-way ANOVA or Student *t* test with α-level of 0.05 (two tailed). In all cases a normality test and equal variance test were performed before the ANOVA to ensure its validity. Post hoc Bonferroni or Duncan's tests were used where appropriate. Data are shown as mean ± SEM.

ACKNOWLEDGMENTS. The authors thank Brenda Takabe and the Division of Comparative Medicine staff for their technical assistance.This work was supported by a Canadian Institute of Health Research grant, National Institute on Drugs of Abuse Grant DA23597, and Tobacco Related Disease Research Program of the State of California Grant 12RT-0099 and the Pearson Center for Alcoholism and Addiction Research. This is publication number 21594 from The Scripps Research Institute.

1. Fellows JL, Trosclair A, Adams EK, Rivera CC (2002) Annual smoking-attributable mortality, years of potential life lost, and economic costs—United States, 1995–1999. *Morb Mortal Wkly Rep* 51:300–303.
2. Koob GF, Le Moal M (2006) *Neurobiology of Addiction* (Elsevier Inc., San Diego), 1st Ed.
3. George O, et al. (2007) CRF-CRF1 system activation mediates withdrawal-induced increases in nicotine self-administration in nicotine-dependent rats. *Proc Natl Acad Sci USA* 104:17198–17203.
4. Kalivas PW, Volkow ND (2005) The neural basis of addiction: A pathology of motivation and choice. *Am J Psychiatry* 162:1403–1413.
5. Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat Neurosci* 6:968–973.
6. Goto Y, Grace AA (2005) Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat Neurosci* 8:805–812.
7. Grace AA (2000) The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. *Addiction* 95(Suppl 2):S119–S128.
8. Grunberg NE (1994) Overview: Biological processes relevant to drugs of dependence. *Addiction* 89:1443–1446.
9. Laviolette SR, van der Kooy D (2004) The neurobiology of nicotine addiction: Bridging the gap from molecules to behaviour. *Nat Rev Neurosci* 5:55–65.
10. Nestler EJ (2005) Is there a common molecular pathway for addiction? *Nat Neurosci* 8: 1445–1449.
11. Fowler CD, Lu Q, Johnson PM, Marks MJ, Kenny PJ (2011) Habenular α5 nicotinic receptor subunit signalling controls nicotine intake. *Nature* 471:597–601.
12. Laviolette SR, Alexson TO, van der Kooy D (2002) Lesions of the tegmental pedunculopontine nucleus block the rewarding effects and reveal the aversive effects of nicotine in the ventral tegmental area. *J Neurosci* 22:8653–8660.
13. Hikida T, Kimura K, Wada N, Funabiki K, Nakanishi S (2010) Distinct roles of synaptic transmission in direct and indirect striatal pathways to reward and aversive behavior. *Neuron* 66:896–907.
14. Zweifel LS, et al. (2009) Disruption of NMDAR-dependent burst firing by dopamine neurons provides selective assessment of phasic dopamine-dependent behavior. *Proc Natl Acad Sci USA* 106:7281–7288.
15. Mameli-Engvall M, et al. (2006) Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. *Neuron* 50:911–921.
16. Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. *Nat Neurosci* 7:583–584.
17. Zhang T, et al. (2009) Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *J Neurosci* 29:4035–4043.
18. Tan H, Bishop SF, Lauzon NM, Sun N, Laviolette SR (2009) Chronic nicotine exposure switches the functional role of mesolimbic dopamine transmission in the processing of nicotine's rewarding and aversive effects. *Neuropharmacology* 56:741–751.
19. Sotak BN, Hnasko TS, Robinson S, Kremer EJ, Palmiter RD (2005) Dysregulation of dopamine signaling in the dorsal striatum inhibits feeding. *Brain Res* 1061:88–96.
20. Grieder TE, et al. (2010) Dopaminergic signaling mediates the motivational response underlying the opponent process to chronic but not acute nicotine. *Neuropsychopharmacology* 35:943–954.
21. Laviolette SR, Nader K, van der Kooy D (2002) Motivational state determines the functional role of the mesolimbic dopamine system in the mediation of opiate reward processes. *Behav Brain Res* 129:17–29.
22. Grace AA, Bunney BS (1983) Intracellular and extracellular electrophysiology of nigral dopaminergic neurons—1. Identification and characterization. *Neuroscience* 10: 301–315.
23. George O, Grieder TE, Cole M, Koob GF (2010) Exposure to chronic intermittent nicotine vapor induces nicotine dependence. *Pharmacol Biochem Behav* 96:104–107.
24. Rahman S, Zhang J, Engleman EA, Corrigall WA (2004) Effects of acute and chronic nicotine on somatodendritic dopamine release of the rat ventral tegmental area: in vivo microdialysis study. *Neuroscience* 129:415–424.
25. Cheer JF, Marsden CA, Kendall DA, Mason R (2000) Lack of response suppression follows repeated ventral tegmental cannabinoid administration: An in vitro electrophysiological study. *Neuroscience* 99:661–667.
26. Cheer JF, et al. (2007) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci* 27:791–795.
27. Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P (2002) SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* 13:451–463.
28. Schilström B, et al. (2004) Nitric oxide is involved in nicotine-induced burst firing of rat ventral tegmental area dopamine neurons. *Neuroscience* 125:957–964.
29. Tozzi A, et al. (2011) The distinct role of medium spiny neurons and cholinergic interneurons in the D_2/A_2A receptor interaction in the striatum: implications for Parkinson's disease. *J Neurosci* 31:1850–1862.
30. Fuxe K, et al. (2010) Adenosine-dopamine interactions in the pathophysiology and treatment of CNS disorders. *CNS Neurosci Ther* 16:e18–e42.
31. Tanganelli S, et al. (2004) Striatal plasticity at the network level. Focus on adenosine A2A and D2 interactions in models of Parkinson's Disease. *Parkinsonism Relat Disord* 10:273–280.
32. Vargas-Perez H, et al. (2009) Ventral tegmental area BDNF induces an opiate-dependent-like reward state in naive rats. *Science* 324:1732–1734.
33. Merritt LL, Martin BR, Walters C, Lichtman AH, Damaj MI (2008) The endogenous cannabinoid system modulates nicotine reward and dependence. *J Pharmacol Exp Ther* 326:483–492.
34. Fehr C, et al. (2008) Association of low striatal dopamine d2 receptor availability with nicotine dependence similar to that seen with other drugs of abuse. *Am J Psychiatry* 165:507–514.
35. Volkow ND, Fowler JS, Wang GJ, Baler R, Telang F (2009) Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology* 56(Suppl 1):3–8.
36. David V, Besson M, Changeux JP, Granon S, Cazala P (2006) Reinforcing effects of nicotine microinjections into the ventral tegmental area of mice: Dependence on cholinergic nicotinic and dopaminergic D1 receptors. *Neuropharmacology* 50: 1030–1040.
37. Lintas A, et al. (2011) Identification of a dopamine receptor-mediated opiate reward memory switch in the basolateral amygdala-nucleus accumbens circuit. *J Neurosci* 31: 11172–11183.
38. Natividad LA, Tejeda HA, Torres OV, O'Dell LE (2010) Nicotine withdrawal produces a decrease in extracellular levels of dopamine in the nucleus accumbens that is lower in adolescent versus adult male rats. *Synapse* 64:136–145.
39. Castañé A, Soria G, Ledent C, Maldonado R, Valverde O (2006) Attenuation of nicotine-induced rewarding effects in A2A knockout mice. *Neuropharmacology* 51: 631–640.
40. Laviolette SR, van der Kooy D (2003) Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. *Mol Psychiatry* 8:50–59, 9.
41. Chausmer AL, Katz JL (2002) Comparison of interactions of D1-like agonists, SKF 81297, SKF 82958 and A-77636, with cocaine: Locomotor activity and drug discrimination studies in rodents. *Psychopharmacology (Berl)* 159:145–153.
42. Ralph RJ, Caine SB (2005) Dopamine D1 and D2 agonist effects on prepulse inhibition and locomotion: comparison of Sprague-Dawley rats to Swiss-Webster, 129X1/SvJ, C57BL/6J, and DBA/2J mice. *J Pharmacol Exp Ther* 312:733–741.
43. Fontinha BM, et al. (2009) Adenosine A2A receptor modulation of hippocampal CA3-CA1 synapse plasticity during associative learning in behaving mice. *Neuropsychopharmacol* 34:1865–1874.
44. Le Foll B, Goldberg SR (2004) Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* 15:2139–2143.