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Chronic treatment with the vasopressin 1b receptor antagonist SSR149415 prevents the dysphoria associated with nicotine withdrawal in rats

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Abstract

Nicotine addiction is a chronic brain disorder that is characterized by dysphoria upon smoking cessation and relapse after brief periods of abstinence. It has been hypothesized that the negative mood state associated with nicotine withdrawal is partly mediated by a heightened activity of brain stress systems. Animal studies suggest that blockade of vasopressin 1b (V1b) receptors diminishes high levels of drug intake in dependent animals and attenuates the emotional response to stressors. The goal of the present studies was to investigate the effect of acute and chronic treatment with the V1b receptor antagonist SSR149415 on the negative mood state associated with nicotine withdrawal in rats. An intracranial self-stimulation (ICSS) procedure was used to assess mood states and nicotine dependence was induced using minipumps. The nicotinic receptor antagonist mecamylamine was used to precipitate withdrawal. Mecamylamine elevated the brain reward thresholds of the nicotine dependent rats, which reflects a negative mood state. Mecamylamine did not affect the brain reward thresholds of the saline-treated control rats. Chronic treatment with SSR149415 completely prevented the elevations in brain reward thresholds associated with nicotine withdrawal while acute treatment only partly prevented nicotine withdrawal. These data suggest that chronic treatment with V1b receptor antagonists may prevent the dysphoria associated with smoking cessation and thereby improve relapse rates.

Keywords

Nicotine; withdrawal; ICSS; dysphoria; vasopressin 1b receptor; SSR149415

1. Introduction

Nicotine addiction is a chronic brain disorder that is characterized by dysphoria upon smoking cessation and relapse after periods of abstinence [1]. The World Health

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Organization estimates that there are one billion smokers worldwide and that about 6 million people die each year from smoking or second hand smoke exposure [2]. Smoking has detrimental effects on human health and increases the risk for cancer, cardiovascular disorders, chronic obstructive pulmonary disease, and dementia and Alzheimer's disease [3-5].

The acute rewarding and cognitive enhancing effects of nicotine play an important role in the initiation of smoking [1, 6]. However, it has been suggested that negative mood states play an important role in the transition from experimenting with cigarettes to high levels of smoking and in the maintenance of smoking and relapse [7]. This is supported by the observation that people who are trying to quit smoking are most likely to relapse during the first week when the negative affective withdrawal symptoms are most severe [8, 9]. People with depression are more likely to relapse than people without a mood disorder [10, 11]. Furthermore, preclinical studies with cocaine indicate that animals that display the greatest deficit in reward function during withdrawal display the greatest increase in drug intake over time [12]. Therefore, it is critical to understand the neuronal mechanisms that mediate the negative mood state associated with smoking cessation.

Animal models have been developed to study the negative mood state associated with smoking cessation. In particular, the intracranial self-stimulation (ICSS) procedure has been widely used to study the effects of drugs of abuse on brain reward function [13-15]. Acute administration of drugs of abuse lowers ICSS / brain reward thresholds which is indicative of a potentiation of brain reward function. In contrast, cessation of chronic drug administration leads to elevations in brain reward thresholds, which is indicative of a negative mood state. Previous studies have shown that acute nicotine administration lowers brain reward thresholds and that the administration of nicotinic receptor (nAChR) antagonists to nicotine dependent rats or cessation of nicotine administration leads to elevations in brain reward thresholds [16, 17]. The US Food and Drug Administration (FDA)-approved smoking cessation drugs bupropion (Zyban®) and varenicline (Chantix®) prevent the elevations in brain reward thresholds associated with nicotine withdrawal in rats [17, 18]. Therefore, this animal model can be used to identify new treatments that diminish nicotine withdrawal and improve relapse rates in humans.

Preclinical studies point to a critical role for brain stress systems in nicotine addiction [19]. Nicotine withdrawal increases the release of CRF in the brain and drugs that block the CRF type 1 (CRF_1) receptor prevent the negative mood state associated with nicotine withdrawal [20, 21]. Recent studies also point to a critical role for vasopressin in regulating mood states [22]. Vasopressin is expressed in brain sites that regulate mood states such as the paraventricular nucleus of the hypothalamus, bed nucleus of the stria terminalis, and the lateral septum [23, 24]. Plasma vasopressin levels and vasopressin mRNA levels in the hypothalamus are increased in people with depression and antidepressants decrease vasopressin mRNA levels in the hypothalamus of rats with high levels of anxiety [25-27]. Vasopressin mediates its effects via the activation of the vasopressin type 1a receptor (V1a), V1b receptor, and V2 receptor, but there is extensive evidence that the effects of stress on mood states are mediated via the V1b receptor [22, 28]. The role of vasopressin and the V1b receptor in nicotine addiction is rather unexplored, but studies with other drugs of abuse

suggest that the V1b receptor plays a role in drug addiction. The V1b receptor antagonist SSR149415 decreases alcohol intake in Sardinian alcohol-preferring rats and in alcohol-dependent rats with high levels of alcohol intake but does not affect alcohol intake in animals that are not dependent and have low levels of alcohol intake [29, 30]. Furthermore, cessation of high levels of cocaine administration to rats leads to the release of adrenocorticotropic hormone (ACTH) and corticosterone and this is attenuated by V1b receptor blockade [31]. Several studies have shown that chronic but not acute administration of a V1b receptor antagonist has antidepressant-like effects [32, 33]. This would suggest that long-term blockade of V1b receptors induces adaptations that provide protection against the aversive effects of stress or drug withdrawal.

The goal of the present studies was to investigate the effects of acute and chronic treatment with the V1b receptor antagonist SSR149415 (V1a Ki 613 ± 144; V1b Ki 1 ± 0.3; V2 Ki 160 ± 26; Oxytocin receptor Ki 40 ± 4) on the negative mood state associated with nicotine withdrawal [34]. Somatic nicotine withdrawal signs are very mild in humans and relapse to smoking is more likely due to negative affective withdrawal signs than somatic signs [1, 7]. Therefore, the present studies will focus on negative affective signs associated with nicotine withdrawal. The rats were prepared with ICSS electrodes in the lateral hypothalamus to assess mood states. In addition, response latencies were assessed to determine whether the V1b antagonist has sedative effects or induces motor impairments. Nicotine dependence was induced using minipumps and withdrawal was precipitated with the nonselective and noncompetitive nicotinic acetylcholine receptor (nAChR) antagonist mecamylamine [35]. The present studies suggest V1b receptor blockade might be a potential new treatment for the dysphoria associated with smoke cessation.

2. Material and Methods

2.1. Animals

Male Wistar rats (Charles River, Raleigh, NC, USA) weighing 250-300 g at the beginning of the experiments were used. The rats were housed with two per cage in a climate-controlled vivarium and maintained on a 12 h reversed light-dark cycle (lights off at 9 AM). The ICSS training sessions and testing were done during the first 4 h of the dark cycle. All subjects were treated in accordance with the National Institutes of Health guidelines 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 (AAALAC) and approved by the University of Florida Institutional Animal Care and Use Committee.

2.2. Drugs

Nicotine, mecamylamine, and pentobarbital were purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in sterile saline (0.9% sodium chloride). The V1b receptor antagonists SSR149415 ((2*S*,4*R*)-1-[5-chloro-1-[(2,4-dimethoxyphenyl)sulfonyl]-3-(2-methoxy-phenyl)-2-oxo-2,3- dihydro-1*H*-indol-3-yl]-4-hydroxy-*N*,*N*-dimethyl-2-pyrrolidine carboxamide, isomer (−); Sanofi, Paris, France)[36] was dissolved in a mixture of dimethyl

sulfoxide (DMSO) (5% v/v), Cremophor EL (5% v/v), and saline. Drug doses are expressed as salt with the exception of the nicotine dose which is expressed as free base.

2.3. Experimental design

In the first experiment, the effect of vehicle and 3 doses of SSR149415 (0.1, 0.5, 2 μg, intracerebroventricular [icv]) on mecamylamine-precipitated nicotine withdrawal was investigated. Rats (n=28) were prepared with ICSS electrodes in the brain, and when the brain reward thresholds were stable (less than 10% variation within a 5 day period) they were prepared with saline (n=12) or nicotine (n=16) pumps. At least 6 days later, the effect of SSR149415 on mecamylamine-precipitated nicotine withdrawal was investigated. There was a 6-day period between the implantation of the minipumps and the start of the mecamylamine injections to allow the development of dependence. The V1b receptor antagonist was administered 25 min before the administration of mecamylamine (3 mg/kg, subcutaneous [sc]), and 10 min after the administration of mecamylamine the rats were placed in the operant chambers to assess brain reward thresholds and response latencies. The pretreatment interval of SSR149415 was based on previous studies that reported behavioral and neuroendocrine effects 30 min after administration [36-38]. Furthermore, mecamylamine elevates the brain reward thresholds of nicotine dependent rats when administered 5-10 min before ICSS testing [21, 39]. The V1b antagonist SSR149415 was administered according to a Latin square design and there was a 4-day wash-out period between subsequent injections. This is sufficient time for the brain reward thresholds to return to baseline levels as the plasma half-life of mecamylamine is 1.2 h [40]. During the wash-out periods the rats were tested daily in the ICSS procedure. In the second experiment, the effect of chronic pretreatment with SSR149415 (0.5 μg per day for 6 days, icv) on nicotine withdrawal was investigated. Rats (n=38) were prepared with ICSS electrodes and when the brain reward thresholds were stable they received saline or nicotine pumps. About half the rats in each group received SSR149415 (saline-SSR149415 n=11, nicotine-SSR149415 n=10) and the other half received vehicle (saline-vehicle n=9, nicotine-vehicle n=8). The rats received one icv infusion per day and the first 5 infusions were given 1 h after the ICSS test sessions. The 6^{th} and final infusion was given 25 min before the administration of mecamylamine (3 mg/kg, sc) and 10 min later the rats were placed in the operant chambers.

2.4. Cannula and electrode implantations

The rats were anesthetized with an isoflurane/oxygen vapor mixture and placed in a stereotaxic frame. The rats were then prepared with a cannula above the lateral ventricle and an electrode in the lateral hypothalamus / medial forebrain bundle as described previously [21, 41, 42]. The cannulas were implanted 2.5 mm above the lateral ventricle using the following flat skull coordinates: anterior posterior (AP) −0.9 mm, medial lateral (ML) ±1.4 mm, dorsal ventral (DV) −3.0 mm from dura. The electrodes were implanted in the lateral hypothalamus using the following coordinates: AP −0.5 mm, ML ±1.7 mm, DV −8.3 mm from dura (incisor bar 5 mm above interaural line). The cannulas and electrodes were secured with skull screws (Plastics One, Roanoke, VA, USA) and dental cement (Co-Oral-Ite Dental, Diamond Springs, CA, USA). When the studies were completed the rats were euthanized with an overdose of pentobarbital (150 mg/kg, ip), and cannula placements were

verified by administering 5 μL of a 0.5% aqueous methyl blue solution at the injection site [21].

2.5. Osmotic minipump implantations

The rats were prepared with osmotic minipumps (model 2ML4, 28 day pumps, Durect Corporation, Cupertino, CA, USA) filled with either saline or nicotine. The pumps were implanted subcutaneously under isoflurane/oxygen anesthesia. The nicotine concentration was adjusted to compensate for differences in body weight and to deliver 3.16 mg/kg/day of nicotine base per day. Chronic administration of nicotine induces tolerance to the effects of nicotine and therefore continuous administration of nicotine does not affect baseline brain reward thresholds or response latencies in the ICSS procedure [21, 43].

2.6. Intracranial self-stimulation procedure

Rats were trained on a modified discrete-trial ICSS procedure [44], as described previously [21, 45]. The rats were tested in twelve operant conditioning chambers that are placed in sound-attenuating chambers (Med Associates, Georgia, VT, USA). After recovery from the intracranial surgeries, the rats were trained to turn a response wheel on a fixed-ratio (FR) 1 schedule of reinforcement. Each quarter turn of the wheel resulted in the delivery of a 0.5 s train of 0.1 ms cathodal square-wave pulses at a frequency of 100 Hz. After the acquisition of responding (100 reinforcements within 10 min), each trial began with the delivery of a non-contingent electrical stimulus, followed by a 7.5 s response window during which the animal could respond to receive a second contingent stimulus that was identical to the initial stimulus. A response during this 7.5 s window was labeled a positive response and the lack of a response was labeled a negative response. During the 2 s period immediately after a positive response, additional responses had no consequences. The inter-trial interval (ITI) that followed a positive response or the end of the response window (in the case of a negative response) had an average duration of 10 s. Responses that occurred during the ITI resulted in a further 12.5 s delay of the onset of the next trial. During the training period, the duration of the ITI and delay periods induced by time-out responses were gradually increased until animals performed consistently. Then brain reward thresholds were assessed by using a modification of the psychophysical method of limits. Test sessions consisted of four alternating series of descending and ascending current intensities starting with a descending series. Blocks of three trials were presented to the rats at a given stimulation intensity, and the intensity was altered systematically between blocks of trials by 5 μA steps. The initial stimulus intensity was set 40 μA above the baseline current-threshold for each animal. Each test session typically lasted 30 min and provided two variables: brain reward thresholds and response latencies. The brain reward threshold was defined as the midpoint between stimulation intensities that supported responding (i.e., positive responses on at least two of the three trials) and stimulation intensities that failed to support responding. Four threshold estimates were recorded and the mean of these values was taken as the final threshold. The time interval between the beginning of the non-contingent stimulus and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency on all trials during which a positive response occurred.

2.7. Statistical analyses

In the first and second experiment, baseline ICSS parameters (5-day averages, brain reward thresholds and response latencies) prior to minipump implantation were compared using one-way analysis of variance (ANOVA). The ICSS parameters during chronic nicotine or saline administration (days 1-6) were expressed as a percentage of the pre-pump implantation values (5-day average). These ICSS parameters were analyzed using two-way repeated measures ANOVAs with time as the within-subjects factor and pump content (nicotine vs. saline) as the between-subjects factor.

In the first experiment, ICSS parameters during the withdrawal tests were expressed as a percentage of the pre-test day baseline values and analyzed using two-way ANOVAs with pump content as the between-subjects factor and drug treatment (dose of SSR1494150) as the within-subjects factors. Absolute pre-test day brain reward thresholds and response latencies were compared using repeated measures one-way ANOVAs. In the second experiment, ICSS parameters during chronic SSR149415 or vehicle administration and during the withdrawal tests were expressed as a percentage of the pre-pump implantation values (5-day average). The effects of chronic administration of SSR149415 on ICSS parameters prior to the withdrawal phase were analyzed using three-way repeated measures ANOVAs with time (4 days, rats received SSR149415 for a total of 6-days but the drug was administered after the first ICSS session and the sixth session was the withdrawal test-day) as the within-subjects factor and drug (SSR149415 vs. vehicle) and pump content as the between-subjects factors. The effects of chronic SSR149415 on ICSS parameters during withdrawal were analyzed using two-way ANOVAs with pump content and drug treatment as the between-subjects factors.

Statistically significant results in the ANOVAs were followed by Bonferroni post hoc comparisons. The data were analyzed with GraphPad Prism version 6 and IBM SPSS Statistics version 22. Probability values less than 0.05 were considered significant.

3. Results

3.1. Acute SSR149415 administration partly prevents the negative mood state associated with nicotine withdrawal

Before the implantation of the minipumps there were no differences in brain reward thresholds or response latencies between the saline and the nicotine group. Furthermore, there was no difference in response latencies between the nicotine and saline rats after the implantation of the minipumps (day 1-6). After the implantation of the minipumps there was a small (~10%), but significant, increase in the brain reward thresholds of the nicotine and saline rats (Time: $F_{5,130}=3.39$, $P<0.01$). Repeated administration of SSR149415 did not affect the absolute pre-test day brain reward thresholds and response latencies (table 1). The administration mecamylamine elevated the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated control rats (Pump: $F_{1,26}=182.0$, $P< 0.0001$, Figure 1). Pretreatment with the V1b receptor antagonist SSR149415 attenuated the elevations in brain reward thresholds associated with nicotine withdrawal and did not affect the brain reward thresholds of the saline-treated control rats

(Dose: F3,78=2.98, P<0.05). The post hoc analyses indicated that 0.1, 0.5, and 2 μg of SSR149415 diminished the elevations in brain reward thresholds in the nicotine withdrawing rats. However, the brain reward thresholds of the nicotine withdrawing rats treated with SSR149415 remained above baseline levels and thus the V1b antagonist did not completely prevent the negative mood state associated with nicotine withdrawal. Neither mecamylamine nor SSR149415 affected the response latencies of the saline-treated control rats.

3.2. Chronic SSR149415 administration completely prevents the negative mood state associated with nicotine withdrawal

Before the implantation of the minipumps there were no differences in brain reward thresholds or response latencies between the experimental groups (saline-vehicle, saline-SSR149415, nicotine-vehicle, and nicotine-SSR149415). Chronic administration of nicotine or saline did not affect the brain reward thresholds or response latencies. Furthermore, chronic administration of SSR149415 did not affect the brain reward thresholds or response latencies of the nicotine and saline-treated rats (Table 2). The nAChR antagonist mecamylamine elevated the brain reward thresholds of the nicotine-treated rats and did not affect the brain reward thresholds of the saline-treated control rats (Pump: F1,34=19.73, P<0.0001, Figure 2). Chronic treatment with the V1b receptor antagonist SSR149415 prevented the elevations in brain reward thresholds associated with nicotine withdrawal and did not affect the brain reward thresholds of the saline-treated control rats (Drug: F1,34=4.39, P<0.05; Pump x Drug interaction: F1,34=4.79, P<0.05). The post hoc comparisons showed that the brain reward thresholds of the nicotine-treated rats that received SSR149415 were lower than those of the nicotine-treated rats that received vehicle. Furthermore, there were no difference in brain reward thresholds between the nicotine-SSR149415 rats and saline-vehicle rats, thus indicating that SSR149415 completely prevented the elevations in brain reward thresholds associated with nicotine withdrawal. The administration of mecamylamine, SSR149415, or both drug together did not affect the response latencies of the nicotine and saline-treated rats. Taken together, this study indicates that chronic treatment with SSR149415 prevents the elevations in brain reward thresholds associated with nicotine withdrawal and does not affect baseline brain reward thresholds or response latencies.

4. Discussion

The goal of the present studies was to investigate the effect of acute and chronic treatment with the V1b receptor antagonist SSR149415 on the negative mood state associated with nicotine withdrawal. As reported previously by our group and others, the nAChR antagonist mecamylamine elevated the brain reward thresholds of the nicotine dependent rats and did not affect the brain reward thresholds of the saline-treated control rats [45, 46]. Acute treatment with SSR149415 partly prevented the negative mood state associated with nicotine withdrawal and chronic treatment with SSR149415 completely prevented the negative mood state associated with nicotine withdrawal. In the saline-treated control animals, acute or chronic treatment with SSR149415 did not affect brain reward function and did not induce sedative effects or motor impairments. The present studies suggest that chronic pretreatment

(~1 week) with a V1b receptor antagonist may prevent the dysphoria associated with smoking cessation.

The present finding that chronic treatment with SSR149415 is more effective than acute treatment in preventing negative mood states is in line with previous studies that investigated the antidepressant-like effects of acute and chronic administration of SSR149415 [32, 33]. Several studies have investigated the effects of SSR149415 on depressive-like behavior using olfactory bulbectomized (OB) rats. Olfactory bulbectomy in rats induces a wide range of behavioral and neurochemical changes that are similar to those in depression and can be reversed by chronic treatment with antidepressants [47]. Iijima and Chaki investigated the effects of acute and chronic treatment with SSR149415 on hyperemotionality in the olfactory bulbectomy model of depression. They showed that acute treatment with SSR149415 does not affect hyperemotionality in OB rats but that chronic treatment with SSR149415 completely prevents hyperemotionality in OB rats [32]. Another study investigated the effects of acute and chronic treatment with SSR149415 on hyperactivity in OB rats. It was shown that acute treatment with SSR149415 does not affect hyperactivity but that chronic treatment with SSR149415 completely prevents hyperactivity [33]. Interestingly, the SSR149415-induced decrease in hyperactivity was even observed 1 week after the cessation of treatment with SSR149415. This pattern of results suggests that chronic treatment with SSR149415 induces neuroadaptations that mediate prolonged antidepressant-like effects.

The present findings support the hypothesis that brain stress systems play a critical role in nicotine withdrawal [1]. Previous studies have provided evidence for a role of the stress peptide CRF in nicotine addiction [19, 20]. Nicotine withdrawal has been associated with an increased release of CRF in the brain and CRF_1 receptor antagonists decrease high levels of nicotine intake after a period of abstinence [20]. In addition, blockade of CRF_1 receptors prevents the negative mood state associated with nicotine withdrawal [39]. Synergistic interactions between CRF and vasopressin have been detected at the level of the hypothalamus. CRF induces the release of ACTH from the anterior pituitary and this effect is potentiated by vasopressin [48, 49]. Chronic stress increases the production of vasopressin in CRF neurons and thereby potentiates the hypothalamic-pituitary-adrenal (HPA) axis response to stress [50, 51]. CRF and vasopressin are also expressed in extrahypothalamic brain sites that play a critical role in the regulation of mood states such as the central nucleus of the amygdala [52]. In addition, it has been shown that vasopressin levels are elevated in the amygdala during heroin withdrawal [53]. In previous studies, it was shown that CRF in the CeA plays a critical role in nicotine withdrawal [39, 45]. Therefore, it might be possible that withdrawal from nicotine leads to increased vasopressin release in the CeA and thereby potentiates the CRF-induced negative mood state.

It is also interesting to note that SSR149415 greatly decreases alcohol intake in alcohol dependent rats with high levels of alcohol intake but does not affect alcohol intake in non-dependent animals with low levels of alcohol intake [29]. Accumulating evidence suggests that low levels of drug intake are mediated by the positive reinforcing (i.e., reward) effects of drugs but that high levels of drug intake are mainly mediated by negative reinforcement processes (drug intake to prevent withdrawal or severe stress)[7]. For example, it has been

shown that in rats with extended access to cocaine the brain reward thresholds are elevated (i.e., dysphoria) between self-administration sessions and drug intake increases of over time [12]. Furthermore, in the aforementioned study there was a positive correlation between the elevations in brain reward thresholds and the escalation of cocaine intake. Thus, animals with the most severe withdrawal symptoms displayed the greatest increase in drug intake over time. The present study showed that blockade of V1b receptors diminishes the negative mood state associated with nicotine withdrawal. Therefore, additional studies are warranted to investigate if blockade of V1b receptors diminishes the escalation of drug intake and the negative mood state that drives the escalation of drug intake.

It cannot be ruled out that non-vasopressinergic mechanisms contributed to the effects of SSR149415 on nicotine withdrawal. For example, it has been reported that SSR149415 modulates cholinergic transmission [54]. Acute and chronic administration of SSR149415 inhibits the release of acetylcholine in the hippocampus of rats [54]. Nicotine withdrawal has been associated with an increased release of acetylcholine in the brain and it has been suggested that the release of acetylcholine might contribute to the aversive aspects of nicotine withdrawal [55]. Therefore, it might be possible that SSR149415 at least partly prevents nicotine withdrawal by diminishing cholinergic transmission in the brain. It has also been reported that drugs that increase serotonergic or noradrenergic transmission attenuate the negative mood state associated with nicotine withdrawal [43, 56]. However, there is currently no evidence that SSR149415 affects serotonin or norepinephrine transmission in the brain [54, 57].

Taken together, the present study shows that acute administration of the V1b receptor antagonist SSR149415 partly prevents the negative mood state associated with nicotine withdrawal and that chronic administration of SSR149415 completely prevents the negative mood state associated with nicotine withdrawal. The present study supports the hypothesis that a dysregulation of brain stress systems contributes to the negative mood state associated with nicotine withdrawal. Therefore, pharmacological treatments that diminish the activity of brain stress systems during a smoking cessation attempt may prevent severe dysphoria and prevent relapse to smoking.

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Highlights

- Nicotine withdrawal induces a negative mood state
- Acute treatment with a V1b receptor antagonist partly prevents withdrawal
- Chronic treatment with a V1b receptor antagonist completely prevents withdrawal
- Blockade of V1b receptors does not affect reward function in control animals
- Blockade of V1b receptors does not induce sedation or motor impairments

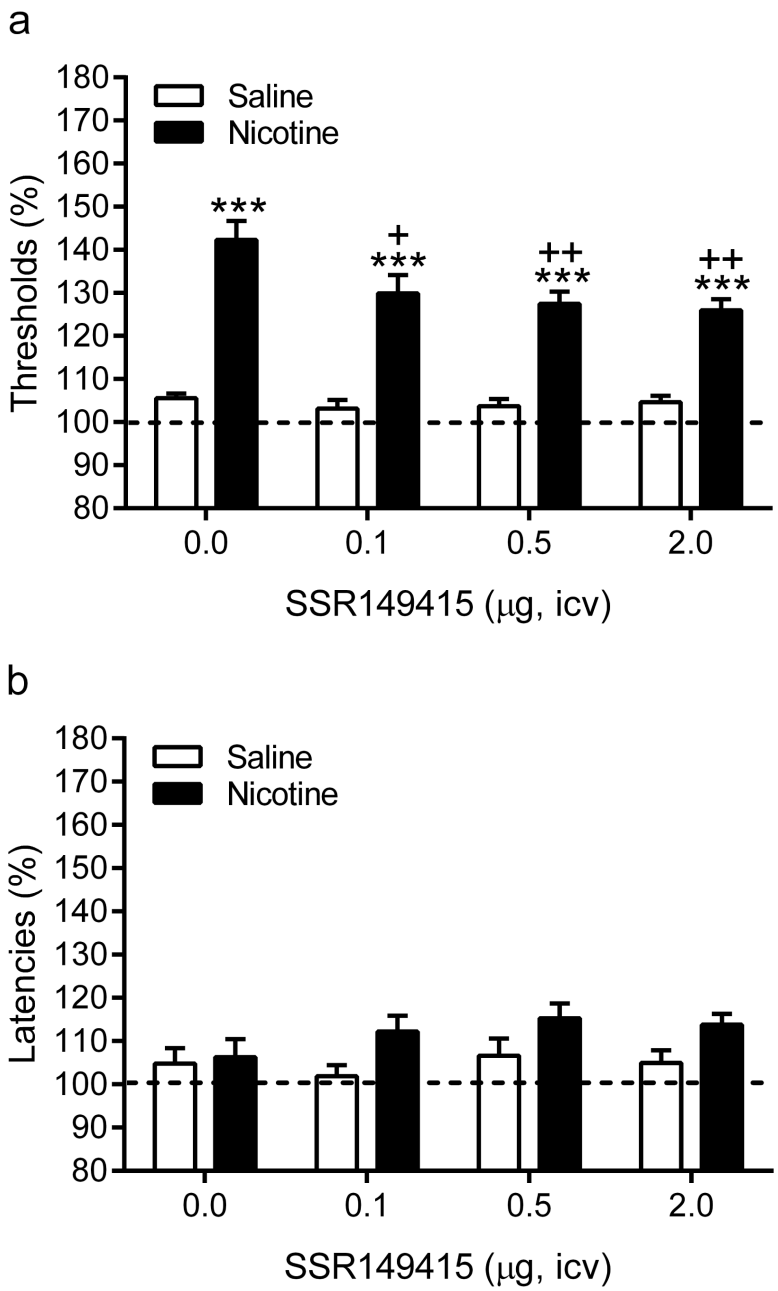

Figure 1.

Acute treatment with SSR149415 partly prevents the negative mood state associated with nicotine withdrawal. Effect of SSR149415 on brain reward thresholds (A) and response latencies (B) during nicotine withdrawal. Asterisks (*** P<0.001) indicate elevated brain reward thresholds compared to the corresponding control group. Plus signs (+ P<0.05, + +P<0.01) indicate lower brain reward thresholds compared to nicotine withdrawing rats that received vehicle (dose 0, icv). Saline n = 12, Nicotine n = 16. Data are expressed as means ± SEM.

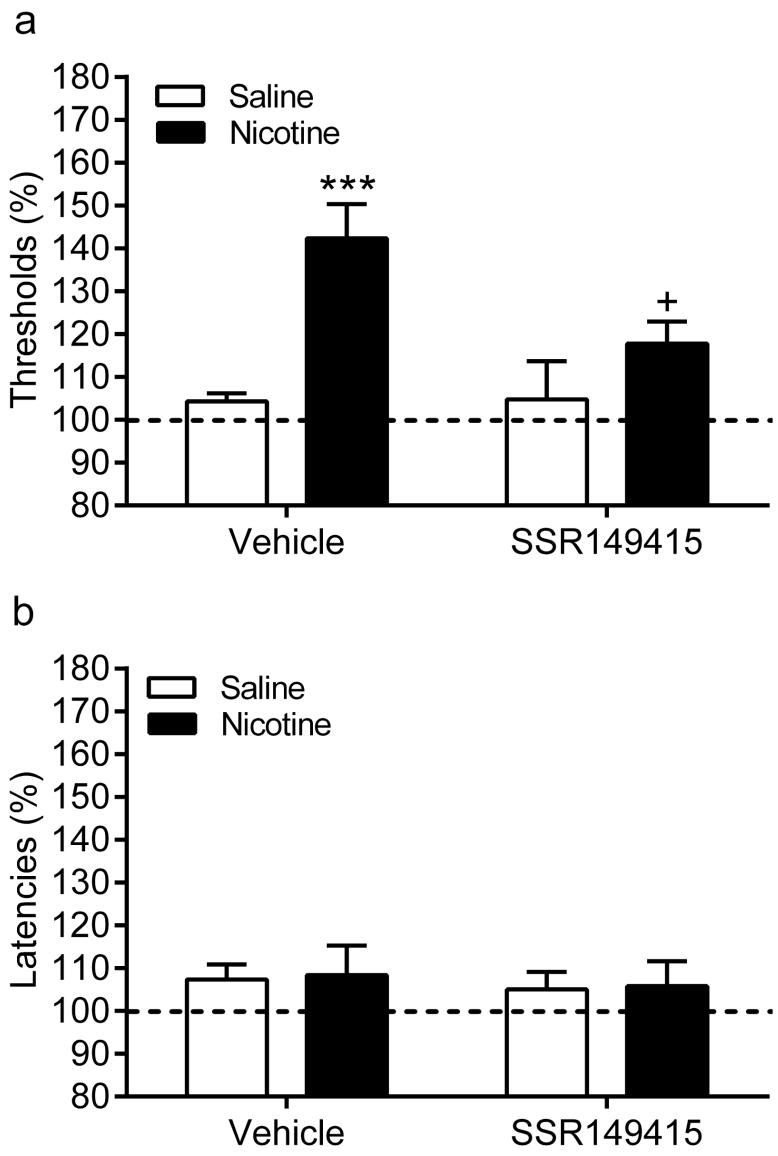

Figure 2.

Chronic treatment with SSR149415 completely prevents the negative mood state associated with nicotine withdrawal. Effect of chronic pretreatment with SSR149415 (0.5 μg) on brain reward thresholds (A) and response latencies (B) during nicotine withdrawal. Asterisks (*** $P<0.001$) indicate elevated brain reward thresholds compared to the corresponding control group. Plus sign (+ $P<0.05$) indicates lower brain reward thresholds compared to the nicotine withdrawing rats that received vehicle (icv). Saline-vehicle n = 9, saline-SSR149415 n=11, nicotine-vehicle n=8, nicotine-SSR149415 n=10. Data are expressed as means ± SEM.

Table 1

Absolute baseline brain reward thresholds and response latencies on pre-test days.

Experimental groups Pump / SSR149415 dose (μg, icv)	**Brain reward thresholds**			
	0	**0.1**	**0.5**	**2**
Saline	124.1 ± 9.3	123.2 ± 6.5	124.9 ± 7.3	128.4 ± 9.1
Nicotine	121.5 ± 7.0	123.4 ± 6.9	123.1 ± 5.5	127.3 ± 6.4
	Latencies			
	0	**0.1**	**0.5**	**2**
Saline	3.3 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.3 ± 0.1
Nicotine	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1	3.2 ± 0.1

Table 2

Effect of the V1b receptor antagonist SSR149415 on baseline brain reward thresholds and response latencies.

Experimental groups	Brain reward thresholds			
	Day 2	Day 3	Day 4	Day 5
Saline-Vehicle	102.7 ± 1.5	100.9 ± 1.8	101.8 ± 2.1	99.6 ± 1.2
Saline-SSR149415	102.3 ± 3.5	99.9 ± 4.3	103.6 ± 6.7	106.2 ± 5.9
Nicotine-Vehicle	103.3 ± 5.5	101.9 ± 5.2	97.9 ± 4.4	100.0 ± 4.1
Nicotine-SSR149415	109.0 ± 5.8	105.9 ± 3.8	108.1 ± 3.0	106.7 ± 3.5
	Latencies			
	Day 2	Day 3	Day 4	Day 5
Saline-Vehicle	99.0 ± 2.7	97.4 ± 2.6	97.8 ± 2.4	103.0 ± 1.6
Saline-SSR149415	96.8 ± 4.4	96.3 ± 1.8	99.5 ± 3.3	100.7 ± 3.2
Nicotine-Vehicle	93.7 ± 2.8	99.5 ± 3.8	96.0 ± 3.1	97.7 ± 4.2
Nicotine-SSR149415	100.0 ± 2.2	97.4 ± 2.6	96.4 ± 2.6	97.0 ± 2.3

The ICSS parameters are expressed as a percentage of pre-pump implantation values (5-day averages). Vehicle or SSR149415 was administered icv after ICSS testing on day 1 (data not shown) and after ICSS testing on days 2-5 (this table), and before the withdrawal session / ICSS testing on day 6 (see Fig. 2).