Brain Stimulation 12 (2019) 1448–1455

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Vagus nerve stimulation during extinction learning reduces conditioned place preference and context-induced reinstatement of cocaine seeking

Jessica E. Childs, Suhyeong Kim, Christopher M. Driskill, Emily Hsiu, Sven Kroener*

School of Behavioral and Brain Sciences, The University of Texas at Dallas, 800 West Campbell Rd., Richardson, TX, 75080, USA

ARTICLE INFO

Article history:
Received 12 March 2019
Received in revised form
26 April 2019
Accepted 1 July 2019
Available online 3 July 2019

Keywords:
Extinction learning
Cue-induced reinstatement
Context-induced reinstatement
Operant conditioning
Vagus nerve stimulation
Craving

ABSTRACT

Background: Drug use causes the formation of strong cue/reward associations which persist long after cessation of drug-taking and contribute to the long-term risk of relapse. Extinguishing these associations may reduce cue-induced craving and relapse. Previously, we found that pairing vagus nerve stimulation (VNS) with extinction of cocaine self-administration reduces cue-induced reinstatement; however, it remains unclear whether this was primarily caused by extinguishing the context, the instrumental response, or both.

Objective: Hypothesis: We hypothesized that VNS can facilitate the extinction of both contextual cues and instrumental responding.

Methods: Extinction of context was first tested using Pavlovian conditioned place preference (CPP). Next, the impact of VNS on the extinction of instrumental responding was assessed under ABA and AAA context conditions. In each extinction context separate groups of rats were either provided the opportunity to perform the instrumental response, or the levers were retracted for the duration of extinction training. Reinstatement was induced by reintroduction of the conditioned stimuli and/or the drug-paired context. Data were analyzed using one-way or two-way repeated measures ANOVAs.

Results: VNS during extinction reduced reinstatement of CPP. VNS also reduced cue- and context-induced reinstatement of the instrumental response under both AAA and ABA conditions. The subjects' ability to engage with the lever during extinction was crucial for this effect. P values < 0.05 were considered significant.

Conclusions: Craving occurs in response to a range of conditioned stimuli and contexts; VNS may improve outcomes of behavioral therapy by facilitating extinction of both an instrumental response and/or contextual cues.

Introduction

During chronic drug use environmental stimuli are repeatedly paired with the effects of the drug, leading to the formation of long-lasting cue/reward associations that can trigger craving and relapse even after extended periods of abstinence [1,2]. Breaking these associations via extinction learning is an important goal in the treatment of substance use disorder (SUD); however, historically extinction-based exposure therapy has not shown the desired long-term success rates [3–5]. Developing therapeutic adjuncts that enhance behavioral treatment is therefore an important goal for SUD research [5–7]. To this end continuing efforts focus on ways to enhance extinction learning and to reverse the maladaptive neuroplasticity that can be seen in animal models of drug use [7–13].

We recently used vagus nerve stimulation (VNS) to enhance the extinction of drug seeking following cocaine self-administration [14]. Animals that received VNS during extinction exhibited reduced cue-induced reinstatement. VNS reduced reinstatement regardless of whether it was tightly paired with the instrumental response (lever pressing) or whether it was delivered at fixed intervals independent of lever pressing. Because cue-induced reinstatement of drug seeking was reduced under both conditions, it is equally possible that VNS functioned to reduce instrumental

Abbreviations: VNS, Vagus nerve stimulation; SUD, Substance use disorder; CS, Conditioned stimulus; CPP, Conditioned place preference.

* Corresponding author.
E-mail address: kroener@utdallas.edu (S. Kroener).

https://doi.org/10.1016/j.brs.2019.07.001
1935-861X/

responding by enhancing the extinction of explicit CS-drug associations, by enhancing the extinction of contextual cues, or both. Considering the strong relationship between re-exposure to drug-associated cues/contexts and relapse [1,6,15], treatments that promote extinction ideally should target both contextual cues and the instrumental response. Therefore, determining whether the effect of VNS is primarily a factor of contextual extinction, extinction of an instrumental response, or ideally both, is important to the translation of these findings into clinical settings. Here we examined the effects of VNS on two critical aspects of extinction: the craving response induced by contextual cues, and drug seeking elicited by explicit CS-response pairings.

The conditioned rewarding and reinforcing properties of drug-paired environmental stimuli can be demonstrated by a) an attraction to drug-paired stimuli in humans and acquired preference for drug-paired contexts in laboratory animals, as seen in conditioned place preference (CPP) experiments [16–18], and b) instrumental responding maintained by CS presentations [19]. Once acquired, incentive motivation for the drug can be measured as non-reinforced instrumental responding, i.e. drug seeking during extinction or "relapse" (drug-, cue-, or context-induced reinstatement). In CPP experiments, drugs of abuse are paired with a specific context and a neutral stimulus (e.g. a saline injection) is paired with a different context. CPP is established when the rewarding properties of the drug cause the drug-paired context to become a conditioned stimulus. This preference can be extinguished by disassociating the context from the reward, usually by repeated exposures of the subject to the space in the absence of drug reward. Drug- or stress-induced reinstatement is then performed to test extinction of the previously drug-paired context. Here we applied VNS during extinction of CPP in order to test whether VNS reduces the craving response to contextual cues acquired during Pavlovian conditioning. We predicted that if VNS facilitated extinction of contextual associations then VNS-treated animals would show reduced CPP during cocaine-induced reinstatement.

Contextual reinstatement (renewal) [20] in ABA drug self-administration paradigms (in which animals self-administer in context A, extinguish in context B, and reinstate in context A) allows to measure what impact the drug-associated environment has on the rate of instrumental responding [21–23]. The rate of the instrumental response is a good analog for the cue reactivity and craving component of SUD [24,25]. To test the effect of VNS paired with extinction specifically on the instrumental response, we used an ABA paradigm in which we trained rats to self-administer cocaine in one context (A). Rats then extinguished either in the same (A) or in a distinct context (B) before they were tested for reinstatement in the original context (A). We predicted that if VNS facilitates the extinction of the instrumental response, rats treated with VNS would show lower response rates during context-induced reinstatement (ABA), similar to what we have previously shown for AAA conditions (in which animals self-administer, extinguish, and reinstate in a single context, A). We further isolated the effect of VNS on the instrumental response by making levers available to some groups of rats during extinction, but not others. We predicted that rats that extinguished in a different context (B) and also were given no opportunity to extinguish the instrumental response (i.e. no levers present) would not benefit from VNS-induced synaptic plasticity and would thus display reinstatement rates comparable to sham-stimulated rats. On the other hand, in rats that had no access to levers but extinguished in the same context (A) reduced drug seeking during reinstatement would suggest an influence of VNS on the extinction of contextual cues. Our results suggest that the ability to perform the instrumental response during extinction was crucial in order for VNS to facilitate extinction learning.

Methods

Subjects

All experiments used male Sprague-Dawley rats (Taconic, Rensselaer, NY) that were at least 60 d old and weighed 250–300 g at the time of surgery. A total of 132 animals were used across all experiments. Each animal participated only in a single type of experiment. Animals were socially housed in an animal care facility on a 12 h reverse light/dark cycle with free access to food and water until the time of surgery. Animals were handled daily for five days before surgery and during the five-day recovery period. Rats used in conditioned place preference experiments were housed individually during recovery and were then socially housed and given free feeding for the remainder of the experiment. Rats used in self-administration experiments were individually housed after surgery and placed on a restricted feeding schedule (25 g/day) of standard rat chow. All protocols were approved by the IACUC of The University of Texas at Dallas and complied with the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Rats were anesthetized with ketamine (85 mg/kg) and xylazine (5 mg/kg). Before the surgery they were pre-treated with atropine (1 mg/kg) to reduce mucosal secretions during surgery, and the local anesthetic bupivicaine at each incision site. For VNS, all animals were implanted with a custom-built cuff electrode placed around the left vagus nerve at the mid-cervical level, with leads connected to a stimulation input site ("headcap") fixed to the skull [26]. Cuff integrity was tested during placement by applying a brief stimulation (0.2 mA, 60 Hz, up to 10 s) of the vagus nerve and noting brief cessation of breathing in the anesthetized rats. Before extinction training, cuff integrity was tested again, using the stimulation parameters applied during extinction training (0.4 mA, 500 μs pulse width at 30 Hz, stimulation cycle of 30 s on), and the response was recorded to an oscilloscope. Waveforms exceeding 10 V were considered an indication of high impedance due to a malfunctioning cuff [26]. If this occurred, animals were excluded from the VNS groups. During the same surgery, animals in the drug self-administration experiments also received a jugular vein catheter implant [14]. To prevent clotting and infection, catheters were flushed daily with a mixture of heparin (0.2 ml of 100 IU) and the antibiotic gentamycin (0.2 ml of 0.1 g/ml). Catheter patency was assessed by infusing 1.0 mg/0.1 ml methohexital sodium. Animals with intact catheters experienced a 5–10 s sedation.

Conditioned place preference

One week after surgery, rats were assessed for their baseline side preference in a place preference apparatus that consisted of two main chambers (31 cm × 31 cm) connected by a small hallway (15 cm × 9 cm). All compartments received ambient illumination from a light source fixed to the inside of the chamber housing. The left side was distinguished by a grid floor and vertical stripes, while the right side had a smooth floor and horizontal stripes (Fig. 1A). Animals were conditioned against their baseline preference by pairing cocaine administration (12 mg/kg I.P.) with a 20-min conditioning session in which they were confined to the chamber opposite their baseline preference. This dose of cocaine lies in the range that produces a large increase of CPP for I.P. injections in male adult rats [27]. Cocaine-conditioning days were balanced with saline-conditioning days, where equal volumes of saline were administered and rats were placed into the chamber opposite the

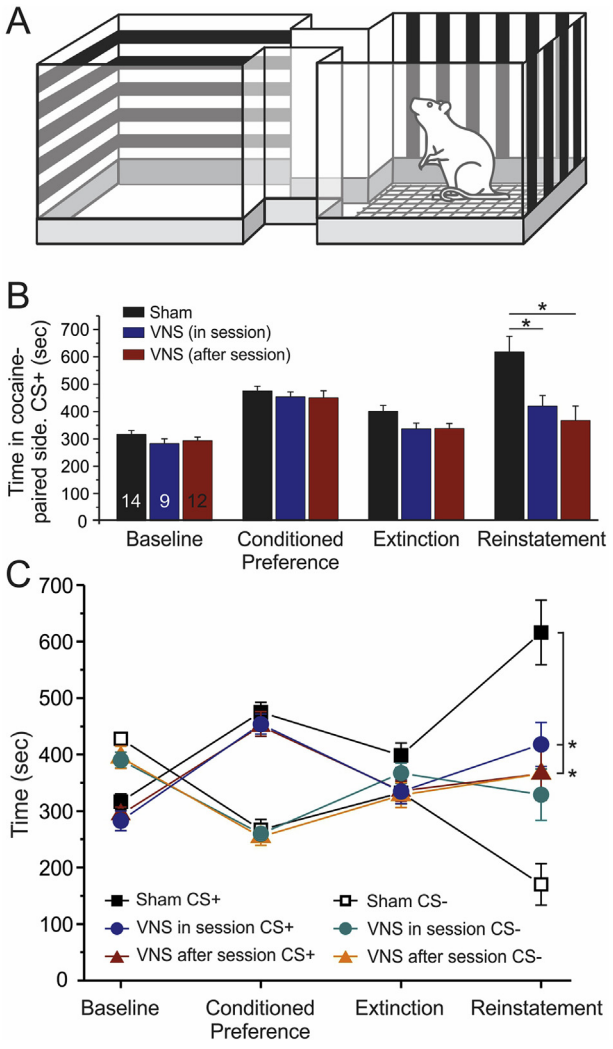

Fig. 1. VNS facilitates extinction of conditioned place preference. A) Illustration of the CPP apparatus, with the left and right compartments distinguished by grid vs. smooth floor and horizontally vs. vertically striped walls. B) Time spent on the cocaine-paired side during the baseline period, the conditioned preference test, the extinction test, and drug-primed reinstatement. Sham-treated animals showed increased preference for the cocaine-paired side compared to animals given VNS during (VNS in session) or shortly after (VNS after session) extinction sessions. C) The graph illustrates how the time spent in the drug-paired side (CS+) and the saline-paired side (CS-) change over the course of the experiment for the three treatment groups. After conditioning, animals in all groups have increased preference for the cocaine-paired side compared to the saline side. Extinction reduces this conditioned preference, but during drug-primed reinstatement only sham-treated animals have a preference for the cocaine-paired side. *P* values are * <0.05.

cocaine-paired side. Conditioning was performed over eight days. On the 9th day, conditioned place preference was assessed by allowing animals access to both sides and recording time spent in each side. The conditioned preference was then extinguished over an additional eight days by alternating exposures to the two sides in the absence of cocaine reward. During all extinction sessions rats were connected to an isolated pulse stimulator (Model 2100, A-M Systems, Carlsborg, WA) via a commutator (RCA, model TP278, Thomson Inc., Socorro, TX) and a soft flexible telephone cable that attached to the leads on their headcaps. Animals tethered in this way showed no signs of restricted movement. On the four extinction days in the formerly cocaine-paired side rats were given either VNS (0.4 mA, 500 μs pulse width at 30 Hz, stimulation cycle of 30 s on and 3 min off), or sham-stimulation designed to short at the level of the headcap for the duration of the 20-min extinction sessions. These VNS parameters are not aversive and do not disrupt ongoing appetitive behavior [14]. Separate cohorts of rats received VNS under one of two conditions: throughout the 20-min extinction sessions (VNS in-session) or for 20 min immediately after the extinction session in their home cage (VNS after-session; stimulation parameters as above, stimulation cycle of 30 s on and 3 min off). On the 18th day, place preference was tested again to determine the effect of extinction training, and on the 19th day a cocaine priming injection (10 mg/kg I.P.) was used to induce reinstatement and preference was measured again.

Drug self-administration and extinction training

One week after surgery, rats were trained in a single overnight session to self-administer food pellets on a fixed ratio 1 (FR1) schedule in an operant conditioning chamber (Med Associates, Saint Albans, VT). Chambers were equipped with two levers, a house light, cue lights over each lever, and a loudspeaker that generated a 2900 Hz tone. Correct lever presses elicited delivery of one food pellet (45 mg, Bio-Serv, Flemmington, NJ). Following the overnight training, animals began daily 2-h drug self-administration sessions during which correct lever presses (left lever, active period indicated by house light) resulted in a cocaine infusion (0.05 ml of 0.5 mg/kg, Sigma, St. Louis, MO). The dose and infusion pattern of cocaine used here produces robust reinforcing effects [28]. Correct lever presses also resulted in the presentation of the correct-lever cue light and tone. Both levers were available for the duration of the session; however, all correct responses were followed by a 20 s timeout during which the house light turned off and lever pressing yielded no response. After at least 10 days of successful drug self-administration (a minimum of 10 correct responses per session), drug seeking behavior was extinguished in one of four conditions: To test the effect of VNS on cue-induced reinstatement, two groups of rats extinguished in the original context (context A). Context A was a standard operant conditioning chamber with a grid floor and clear front wall, back wall, and ceiling (Fig. 3A). During extinction presses at the previous active lever did not result in cocaine delivery or presentation of conditioned stimuli (light and tone). However, only one group of rats had the opportunity to engage in lever pressing (AAA + levers), while for the other group the levers were retracted during the extinction period (AAA -levers), preventing the subjects from engaging in the previously reinforced instrumental response. In order to further test the impact of VNS on context-induced reinstatement, two groups of rats were moved to a distinct operant conditioning chamber (context B) during the extinction period. Assignment of context A or context B as the cocaine self-administration context was counterbalanced across animals. Context B was distinguished by a smooth floor, a striped ceiling, and the presence of a maple scent (Fig. 2A). As for rats in the AAA conditions, one group of animals was allowed to lever press in the chamber (ABA + levers), while for a second group of rats the levers were retracted (ABA -levers). For each of these four conditions rats were further divided into VNS- and Sham-treated groups. During each of the twelve daily 2 h extinction sessions rats in all groups were connected to an isolated pulse stimulator via their headcaps. Rats in the VNS groups received VNS (0.4 mA, 500 μs pulse width at 30 Hz, stimulation cycle of 30 s on and 5 min off), while sham-treated animals were given sham stimulation designed to short at the level of the headcap. After 12 days of extinction training, drug seeking behavior was assessed by placing the animals back into the context in which they previously self-administered cocaine (context A), and nonreinforced

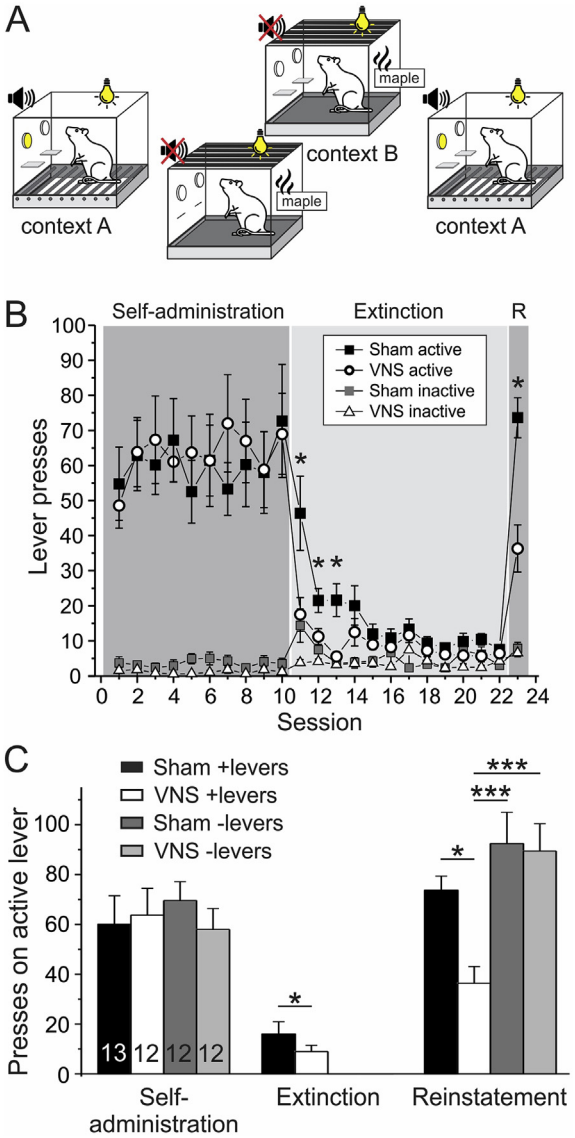

Fig. 2. VNS facilitates extinction of instrumental responding for cocaine and reduces context-induced reinstatement in ABA context conditions. A) Diagram of the experimental setup. Rats self-administered cocaine in context A and extinguished in an alternate context (context B), which was distinguished by a smooth floor (vs. a standard floor grid), the addition of visual cues (stripes), and the addition of a maple scent. For separate groups of rats levers were either extended or retracted during extinction. Re-exposure to the original drug-paired context (context A) was used to drive context-induced reinstatement after extinction. B) Response rates at active- and inactive levers during self-administration, extinction, and context-induced reinstatement in those groups of rats that had access to levers during extinction (ABA Sham + levers, ABA VNS + levers). Sham and VNS animals have similar rates of self-administration. During extinction (days 1, 2, and 3) and context-induced reinstatement, Sham-treated animals engage in the instrumental response more than VNS animals do. C) Active lever-presses during self-administration, extinction, and context-induced reinstatement for all rats tested under ABA conditions. Only animals that received VNS during extinction sessions in which levers were available (ABA VNS + levers) show reduced context-induced reinstatement compared to sham-treated animals. *P* values are * <0.05; ** < 0.01; *** < 0.005.

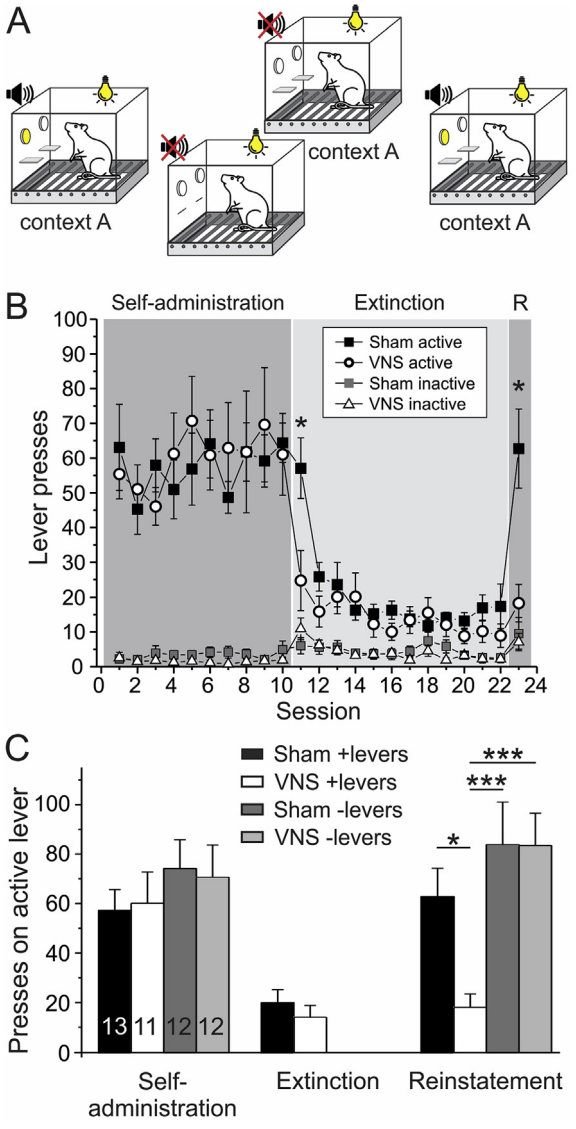

Fig. 3. VNS facilitates extinction of instrumental responding for cocaine and reduces cue-induced reinstatement in AAA context conditions. A) Diagram of the experimental setup. Rats self-administered cocaine and extinguished in the same context. For separate groups of rats levers were either extended or retracted during extinction. Cue-induced reinstatement was achieved by response-contingent presentation of the drug-paired cues. B) Response rates at active- and inactive levers during self-administration, extinction, and cue-induced reinstatement in those groups of rats that had access to levers during extinction (AAA Sham + levers, AAA VNS + levers). Sham-treated and VNS animals have similar rates of self-administration. During reinstatement, sham-treated animals engage in the instrumental response more than VNS animals do. C) Active lever-presses during self-administration, extinction, and cue-induced reinstatement for all rats tested under AAA conditions. Despite the availability of contextual information during extinction, only animals that received VNS during extinction sessions in which levers were available (AAA VNS + levers) have reduced cue-induced reinstatement compared to sham-treated animals. *P* values are * <0.05; ** < 0.01; *** < 0.005.

responding at the levers was assessed in the response-contingent presence of the CS.

Data analysis

Conditioned place preference data were analyzed for each time point (baseline, conditioned preference, extinction, and reinstatement) using a one-way ANOVA with Sidak's post-hoc testing. Drug self-administration data were analyzed using a two-way ANOVA with Sidak's post hoc testing. Extinction data were analyzed using a two-way repeated measures ANOVA. A one-way ANOVA with Sidak's post-hoc testing was used to analyze context-induced reinstatement data. *P* values less than 0.05 were considered significant.

Results

VNS reduces reinstatement of conditioned place preference for cocaine

To determine the effect of VNS on the extinction of contextual associations acquired during Pavlovian conditioning, passive administration of cocaine was repeatedly paired with one side of a two-compartment apparatus and the resultant conditioned place preference was then extinguished in the presence or absence of VNS (Fig. 1A). Rats showed no baseline preference for the cocaine-paired side ($F_{(2,32)} = 1.391$, $p = 0.263$). After 8 days of cocaine-pairings, all groups (sham, $n = 14$; VNS in-session, $n = 9$, VNS after-session, $n = 12$) displayed similar levels of conditioned preference ($F_{(2,32)} = 0.445$, $p = 0.645$). After 8 days of extinction, there was no difference in preference for the cocaine-paired side between sham- and either VNS-treated group ($F_{(2,32)} = 2.814$, $p = 0.077$). In contrast, a one-way ANOVA revealed a main effect of VNS treatment on cocaine-primed reinstatement performed on the following day ($F_{(2,32)} = 6.761$, $p = 0.004$; Fig. 1B and C). Sham-treated animals expressed greater preference for the cocaine-paired side than either of the VNS groups ($Sidak_{[VNS\ in\text{-}session]}$ $p = 0.048$, $n = 9$; $Sidak_{[VNS\ after\text{-}session]}$ $p = 0.004$, $n = 12$). These findings suggest than VNS can reduce contextual associations made during Pavlovian conditioning for place preference.

VNS enhances extinction from drug seeking and reduces both context- and cue-induced reinstatement of cocaine seeking

We previously found that pairing VNS with extinction of drug seeking under AAA context conditions facilitates extinction learning and reduces cue-induced reinstatement of the drug seeking response [14]. To distinguish between VNS' ability to facilitate the extinction of drug-associated contextual cues (as indicated by the results of our CPP experiment) or the instrumental response, we trained rats to self-administer cocaine and then extinguished them under 4 conditions (ABA + levers; ABA −levers; AAA + levers; AAA −levers) while they received VNS or sham-stimulation. Rats in all groups (ABA sham + levers, $n = 13$; ABA VNS + levers, $n = 12$; ABA sham −levers, $n = 12$; ABA VNS −levers, $n = 12$; AAA sham + levers $n = 12$; AAA VNS + levers, $n = 12$; AAA sham -levers, $n = 13$; AAA VNS -levers, $n = 11$) consistently learned to self-administer cocaine, as evidenced by the much higher response rates on the active vs. the inactive lever. A two-way ANOVA analysis of active lever and inactive lever presses across groups during the self-administration phase revealed a main effect of lever assignment ($F_{(1,178)} = 365.6$, $p < 0.0001$) and showed no group effect on response rates for either lever ($F_{(7,178)} = 0.404$, $p = 0.899$). Next, rats extinguished under the conditions outlined above: in the same context (context A) or an alternate context (context B), in the presence or absence of levers, and while they received VNS or sham-stimulation (Fig. 2). In the two groups of rats that extinguished in context B and which had access to levers during extinction (i.e. the groups where a behavioral response could be measured, ABA sham + levers, ABA VNS + levers), a two-way repeated measures ANOVA was used to study the effects of time, treatment, and treatment X time interaction. Animals that received VNS (ABA VNS + levers) showed lower response rates during extinction compared to sham-stimulated rats (ABA sham + levers) ($F_{(1,23)} = 5.552$, $p = 0.027$, Fig. 2B and C). There was also a main effect of time ($F_{(2.483,\ 57.12)} = 11.46$, $p < 0.0001$), as well as a group X time interaction ($F_{(11,253)} = 3.867$, $p < 0.0001$). Twenty-four hours after the last extinction session, rats in all treatment groups were re-exposed to the drug associated context (context A) to assess context-induced reinstatement of the instrumental response. A two-way ANOVA with the variables lever availability and treatment found a main effect of lever availability ($F_{(1,45)} = 13.90$, $p = 0.0005$) and a main effect of treatment ($F_{(1,45)} = 4.365$, $p = 0.0424$). Only those rats which had access to the levers and received VNS during extinction (ABA VNS + levers) showed a reduction in context-induced reinstatement relative to the sham-stimulated control groups ($Sidak_{ABA\ sham\ +levers}$ $p = 0.048$, $n = 13$; $Sidak_{ABA\ sham\ -levers}$ $p = 0.001$, $n = 12$; $Sidak_{ABA\ VNS\ -levers}$ $p = 0.002$, $n = 12$; Fig. 2C), indicating that the ability to engage in the instrumental response during extinction was critical for VNS' ability to modulate drug-associated context-induced reinstatement.

To further explore the idea that VNS' effects on the extinction of drug seeking were primarily driven by the pairing of VNS with discrete events (i.e. non-reinforced lever presses), we replicated our previous findings [14] in two cohorts of rats that self-administered cocaine and extinguished in the same context (AAA), either in the presence or absence of levers (AAA sham + levers, n 12; AAA VNS + levers, $n = 12$; AAA sham −levers, $n = 13$; AAA VNS −levers, $n = 12$). In those groups that had lever access during extinction (AAA sham + levers, AAA VNS + levers; Fig. 3B) drug seeking behavior during extinction was analyzed with a two-way repeated measures ANOVA with the variables time and treatment. For the 12 days of extinction there was a main effect of time ($F_{(3.739,\ 82.26)} = 9.734$, $p < 0.0001$), and a treatment X time interaction ($F_{(11,242)} = 3.215$, $p = 0.0004$). Cue-induced reinstatement was triggered by giving the rats response-contingent access to the CS (and the levers) in the same context. A two-way ANOVA with the variables lever availability and treatment found main effects of both lever availability ($F_{(1,45)} = 16.25$, $p = 0.0002$) and treatment ($F_{(1,45)} = 4.460$, $p = 0.0403$), as well as a lever availability X treatment interaction ($F_{(1,45)} = 4.325$, $p = 0.0433$). Again, only those subjects which had access to the levers and which received VNS during extinction (AAA VNS + levers) showed a reduction in cue-induced reinstatement relative to sham-stimulated controls ($Sidak_{AAA\ sham\ +levers}$ $p = 0.0261$, $n = 13$; $Sidak_{AAA\ sham\ -levers}$ $p = 0.0004$, $n = 13$; $Sidak_{AAA\ VNS\ -levers}$ $p = 0.0008$, $n = 11$; Fig. 3C). Taken together, these findings demonstrate that in rats trained to self-administer cocaine, the ability to perform the instrumental response during extinction is necessary and sufficient for VNS to enhance extinction of drug seeking and to reduce both cue- and context-induced reinstatement.

Discussion

Addiction is a chronic brain disorder in which patients in recovery remain vulnerable to relapse throughout their lifespan. Drug-related cues and contexts create strong memories that trigger craving-in patients [1,2]. One strategy for reducing relapse in human drug addicts is to promote behavioral self-regulation by attempting to extinguish responding to drug-associated environmental stimuli [29,30]. However, this type of exposure therapy for drug addiction has proved only moderately effective in reducing cue-reactivity and thus preventing relapse [29,31]. Therefore, adjunct treatments are desired that can improve both the efficacy and persistence of the effects of exposure therapy [3–7,31]. A range of adjunct treatments have already been tested with varying degrees of success. These include drugs like D-cycloserine [12,32–34], other types of behavioral therapy [3,31], and deep brain stimulation [35,36].

Vagus nerve stimulation is a minimally invasive neuroprosthetic treatment that presents another promising way to induce targeted plasticity [37]. This targeted plasticity has been used to aid in the recovery of sensory and motor function in several disease states [38–41]. Further, VNS has been used to improve memory retention

[42–44]. These findings have prompted research on VNS as a tool to enhance extinction learning. The results from several studies show that VNS facilitates the extinction of both aversive and appetitive stimuli: VNS enhances the extinction of conditioned fear [45,46], even under conditions where extinction learning is normally impaired [47], and it has been used to reduce reinstatement of heroin seeking [48]. Consistent with the latter finding, we recently showed that pairing VNS with extinction from cocaine seeking facilitates extinction learning and reduces cue-induced reinstatement [14]. This effect occurred regardless of whether VNS was delivered contingently with presses on the previously active lever, or at fixed intervals throughout the extinction sessions. The finding that pairing VNS with non-reinforced responses at the previously active lever was sufficient to facilitate both extinction learning and reduce reinstatement suggests that VNS' effect was largely due to extinction of response-outcome associations. However, the fact that non-contingent VNS at fixed intervals was equally effective may imply that at least part of this effect was due to the extinction of contextual associations.

Here we further studied the effects of VNS on extinction by isolating different aspects of contextual- and response-outcome associations, respectively, during extinction. We used conditioned place preference to study the extinction of drug-paired contextual cues following Pavlovian conditioning, and we used variations of ABA and AAA context conditions to study extinction of instrumental responding after cocaine self-administration. Extinction of instrumental responding for drug reinforcers differs from extinction of Pavlovian learning, in part because animals must first be trained on the instrumental response in the test context, and more importantly, because extinction of instrumental responses is an active process that occurs as the extinction context forms an inhibitory association with the instrumental response [49]. In contrast, during Pavlovian conditioning the context acts to gate retrieval of an extinction association [50]. When we used CPP to study extinction of Pavlovian learning, we found that pairing VNS with the extinction of a drug-paired context reduced CPP during drug-induced reinstatement. This effect is unlikely to result from unspecific motor or aversive effects of VNS, as we have previously shown that application of VNS is neither rewarding nor punishing, and does not affect ongoing appetitive behavior towards cocaine or a natural reinforcer [14].

In our studies of extinction of an instrumental response, pairing VNS with extinction in a different context (ABA) also enhanced extinction of drug seeking and reduced context-induced reinstatement (renewal). However, when we attempted to further distinguish the contribution of context and instrumental responses to these effects by altering lever availability under both AAA and ABA context conditions, we found evidence that the subjects' ability to engage in the instrumental response during extinction was an important requirement for VNS' ability to reduce reinstatement. Importantly, the fact that the effects of VNS were similar in the AAA and ABA conditions, respectively, also suggests that any effect of VNS on contextual extinction, as it might be indicated from our CPP experiments, is not sufficient to compete with well-trained instrumental responses. Previous studies using natural reinforcers have shown that the degree of renewal under ABA (or ABC) conditions depends of the strength of associations formed during acquisition. These associations are influenced by the degree of generalization from the extinction context (e.g. B) to the test context (e.g. A) [51]. In our experiments, the A and B contexts were easily distinguishable based on the visual, tactile, and olfactory information provided. Thus, our findings suggest that during conditioning the levers may have acquired discriminative stimulus properties. Therefore, when levers were not available during extinction responding at the active lever was high during reinstatement.

Contextual associations and instrumental responses contribute differently to SUD [1,2,52]. In addition, diverging neural systems mediate cue-induced versus drug-primed incentive motivation for drug and drug reinforcement [53]. The corticolimbic circuits essential for extinction and reinstatement are central to action selection, decision-making, and inhibitory control, and include the prefrontal cortex, the basolateral amygdala, the nucleus accumbens core and shell, the hippocampus, and the ventral tegmental area [50]. We have recently shown that VNS paired with extinction learning alters the expression of the phosphorylated transcription factor cAMP response-element binding protein (pCREB) in a network that includes the medial prefrontal cortex and the basolateral amygdala [14], as well as the nucleus accumbens core and shell [Childs et al., unpublished). The pathway between the medial prefrontal cortex and the amygdala is important for extinction of both drug self-administration and conditioned fear [54], and VNS alters the ability to induce long-term synaptic plasticity in this pathway [14,45]. How VNS facilitates plasticity in the extinction network to facilitate extinction and reduce reinstatement is not well understood, but likely results from increased neurotransmitter release at active synapses. Vagal afferents relay signals to the nucleus tractus solitarius (NTS), which then projects to the parabrachial nucleus, hypothalamus, thalamus, amygdala, and hippocampus [55,56]. Monoamine nuclei in the brainstem and cells in the cholinergic basal forebrain also receive direct and/or indirect projections from the NTS [57–60]. Stimulation of ascending fibers of the cervical vagus nerve therefore leads to the release of several neuromodulators, including norepinephrine, acetylcholine, serotonin, and BDNF [61–67], causing widespread cortical and subcortical activation [68–70], providing a tool for tight temporal and network-specific release of neurotransmitters at physiological concentrations. VNS has been shown to enhance cortical and subcortical plasticity [14,38,39,45,71]. Consistent with the idea that neuromodulators bias the manner in which cortical networks process information [72] this VNS-induced plasticity may act as a primer, modulating synaptic plasticity in response to specific inputs that occur during sensory stimulation [38] or learning and memory [14,39,45,46]. This may explain how VNS broadly augments functions as diverse as memory consolidation [42,43], and motor activity and the subsequent cortical map expansions [39]. The idea that VNS leads to network-specific release of neurotransmitters would also provide insight why in some cases (such as precise sensory discriminations or repeated motor movements) the timing of VNS delivery is very important [73–75], while in other learning situations where multiple complex stimuli have to be integrated over time the effective window can be considerably broader [43,44], but see Ref. [46]. This may explain why in our CPP experiments VNS given immediately after the extinction sessions was equally effective for reducing conditioned place preference as VNS given *during* extinction sessions.

Extinguishing reactivity to drug-associated environments and extinguishing the instrumental response for drugs are both important for the treatment of SUD [1,2,52,76]. What is accomplished in the clinic to reduce cue-reactivity is quickly undone when patients return to their former drug-associated environment [3,15]. Our findings are therefore clinically important for two reasons: First, we show that when paired with VNS, extinction of the instrumental response was similarly facilitated under AAA or ABA context conditions, respectively. When supported with VNS, this extinction learning was robust enough to reduce context-induced reinstatement when the non-extinguished drug-paired context was reintroduced (ABA). Second, while there was some evidence for the extinction of contextual associations in our CPP data, our self-administration experiments tell a different story. Extinction without the opportunity to perform the instrumental response was

not effective in AAA or ABA context conditions, regardless of whether VNS was applied or not. Taken together, these findings further support the potential of VNS as an adjunct treatment during exposure therapy, but they also highlight the need to model specific instrumental responses in these rehabilitative approaches.

Conflicts of interest

No potential conflict of interest is reported by the authors.

Acknowledgments

This work was supported by the National Institutes of Health (1R21 DA043150–01 to SK).

References

[1] Childress A, Ehrman R, McLellan AT, O'Brien C. Conditioned craving and arousal in cocaine addiction: a preliminary report. NIDA Res Monogr 1988;81:74–80.
[2] Ehrman RN, Robbins SJ, Childress AR, O'Brien CP. Conditioned responses to cocaine-related stimuli in cocaine abuse patients. Psychopharmacology 1992;107(4):523–9.
[3] Hammond S, Wagner JJ. The context dependency of extinction negates the effectiveness of cognitive enhancement to reduce cocaine-primed reinstatement. Behav Brain Res 2013;252:444–9.
[4] Taylor JR, Olausson P, Quinn JJ, Torregrossa MM. Targeting extinction and reconsolidation mechanisms to combat the impact of drug cues on addiction. Neuropharmacology 2009;56(Suppl 1):186–95.
[5] Torregrossa MM, Taylor JR. Learning to forget: manipulating extinction and reconsolidation processes to treat addiction. Psychopharmacology 2013;226(4):659–72.
[6] Kantak KM, Nic Dhonnchadha BA. Pharmacological enhancement of drug cue extinction learning: translational challenges. Ann N Y Acad Sci 2011;1216:122–37.
[7] Nic Dhonnchadha BA, Kantak KM. Cognitive enhancers for facilitating drug cue extinction: insights from animal models. Pharmacol Biochem Behav 2011;99(2):229–44.
[8] Hafenbreidel M, Rafa Todd C, Twining RC, Tuscher JJ, Mueller D. Bidirectional effects of inhibiting or potentiating NMDA receptors on extinction after cocaine self-administration in rats. Psychopharmacology 2014;231(24):4585–94.
[9] Moussawi K, Pacchioni A, Moran M, Olive MF, Gass JT, Lavin A, et al. N-Acetylcysteine reverses cocaine-induced metaplasticity. Nat Neurosci 2009;12(2):182–9.
[10] Otis JM, Mueller D. Reversal of cocaine-associated synaptic plasticity in medial prefrontal cortex parallels elimination of memory retrieval. Neuropsychopharmacology 2017;42(10):2000–10.
[11] Otis JM, Fitzgerald MK, Mueller D. Infralimbic BDNF/TrkB enhancement of GluN2B currents facilitates extinction of a cocaine-conditioned place preference. J Neurosci 2014;34(17):6057–64.
[12] Botreau F, Paolone G, Stewart J. d-Cycloserine facilitates extinction of a cocaine-induced conditioned place preference. Behav Brain Res 2006;172(1):173–8.
[13] Malvaez M, Sanchis-Segura C, Vo D, Lattal KM, Wood MA. Modulation of chromatin modification facilitates extinction of cocaine-induced conditioned place preference. Biol Psychiatry 2010;67(1):36–43.
[14] Childs JE, DeLeon J, Nickel E, Kroener S. Vagus nerve stimulation reduces cocaine seeking and alters plasticity in the extinction network. Learn Mem 2017;24(1):35–42.
[15] O'Brien CP, Childress AR, McLellan T, Ehrman R. Integrating systemic cue exposure with standard treatment in recovering drug dependent patients. Addict Behav 1990;15(4):355–65.
[16] Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol 1998;56(6):613–72.
[17] Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 2000;153(1):31–43.
[18] Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. Neurosci Biobehav Rev 1995;19(1):39–51.
[19] Fuchs RA, Bell GH, Ramirez DR, Eaddy JL, Su ZI. Basolateral amygdala involvement in memory reconsolidation processes that facilitate drug context-induced cocaine seeking. Eur J Neurosci 2009;30(5):889–900.
[20] Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci 2008;363(1507):3233–43.
[21] Fuchs RA, Evans KA, Ledford CC, Parker MP, Case JM, Mehta RH, et al. The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. Neuropsychopharmacology 2005;30(2):296–309.
[22] Bouton ME, Todd TP, Vurbic D, Winterbauer NE. Renewal after the extinction of free operant behavior. Learn Behav 2011;39(1):57–67.
[23] Hamlin AS, Clemens KJ, McNally GP. Renewal of extinguished cocaine-seeking. Neuroscience 2008;151(3):659–70.
[24] Carter BL, Tiffany ST. Meta-analysis of cue-reactivity in addiction research. Addiction 1999;94(3):327–40.
[25] Sayette MA, Shiffman S, Tiffany ST, Niaura RS, Martin CS, Shadel WG. The measurement of drug craving. Addiction 2000;95(Suppl 2):S189–210.
[26] Childs JE, Alvarez-Dieppa AC, McIntyre CK, Kroener S. Vagus nerve stimulation as a tool to induce plasticity in pathways relevant for extinction learning. J Vis Exp : JoVE 2015;(102):e53032.
[27] Zakharova E, Wade D, Izenwasser S. Sensitivity to cocaine conditioned reward depends on sex and age. Pharmacol Biochem Behav 2009;92(1):131–4.
[28] Roberts DC, Morgan D, Liu Y. How to make a rat addicted to cocaine. Prog Neuro-Psychopharmacol Biol Psychiatry 2007;31(8):1614–24.
[29] Conklin CA, Tiffany ST. Applying extinction research and theory to cue-exposure addiction treatments. Addiction 2002;97(2):155–67.
[30] Havermans RC, Jansen AT. Increasing the efficacy of cue exposure treatment in preventing relapse of addictive behavior. Addict Behav 2003;28(5):989–94.
[31] Dutra L, Stathopoulou G, Basden SL, Leyro TM, Powers MB, Otto MW. A meta-analytic review of psychosocial interventions for substance use disorders. Am J Psychiatry 2008;165(2):179–87.
[32] Paolone G, Botreau F, Stewart J. The facilitative effects of D-cycloserine on extinction of a cocaine-induced conditioned place preference can be long lasting and resistant to reinstatement. Psychopharmacology 2009;202(1–3):403–9.
[33] Thanos PK, Bermeo C, Wang GJ, Volkow ND. D-cycloserine facilitates extinction of cocaine self-administration in rats. Synapse 2011;65(9):938–44.
[34] Torregrossa MM, Sanchez H, Taylor JR. D-cycloserine reduces the context specificity of pavlovian extinction of cocaine cues through actions in the nucleus accumbens. J Neurosci 2010;30(31):10526–33.
[35] Luigjes J, van den Brink W, Feenstra M, van den Munckhof P, Schuurman PR, Schippers R, et al. Deep brain stimulation in addiction: a review of potential brain targets. Mol Psychiatry 2012;17(6):572–83.
[36] Guercio LA, Schmidt HD, Pierce RC. Deep brain stimulation of the nucleus accumbens shell attenuates cue-induced reinstatement of both cocaine and sucrose seeking in rats. Behav Brain Res 2015;281:125–30.
[37] Hays SA, Rennaker RL, Kilgard MP. Targeting plasticity with vagus nerve stimulation to treat neurological disease. Prog Brain Res 2013;207:275–99.
[38] Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, et al. Reversing pathological neural activity using targeted plasticity. Nature 2011;470(7332):101–4.
[39] Porter BA, Khodaparast N, Fayyaz T, Cheung RJ, Ahmed SS, Vrana WA, et al. Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. Cerebr Cortex 2012;22(10):2365–74.
[40] Hays SA, Khodaparast N, Hulsey DR, Ruiz A, Sloan AM, Rennaker 2nd2nd, et al. Vagus nerve stimulation during rehabilitative training improves functional recovery after intracerebral hemorrhage. Stroke 2014;45(10):3097–100.
[41] Pruitt DT, Schmid AN, Kim LJ, Abe CM, Trieu JL, Choua C, et al. Vagus nerve stimulation delivered with motor training enhances recovery of function after traumatic brain injury. J Neurotrauma 2016;33(9):871–9.
[42] Clark KB, Krahl SE, Smith DC, Jensen RA. Post-training unilateral vagal stimulation enhances retention performance in the rat. Neurobiol Learn Mem 1995;63(3):213–6.
[43] Clark KB, Naritoku DK, Smith DC, Browning RA, Jensen RA. Enhanced recognition memory following vagus nerve stimulation in human subjects. Nat Neurosci 1999;2(1):94–8.
[44] Clark KB, Smith DC, Hassert DL, Browning RA, Naritoku DK, Jensen RA. Post-training electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. Neurobiol Learn Mem 1998;70(3):364–73.
[45] Pena DF, Childs JE, Willett S, Vital A, McIntyre CK, Kroener S. Vagus nerve stimulation enhances extinction of conditioned fear and modulates plasticity in the pathway from the ventromedial prefrontal cortex to the amygdala. Front Behav Neurosci 2014;8:327.
[46] Pena DF, Engineer ND, McIntyre CK. Rapid remission of conditioned fear expression with extinction training paired with vagus nerve stimulation. Biol Psychiatry 2013;73(11):1071–7.
[47] Noble LJ, Gonzalez IJ, Meruva VB, Callahan KA, Belfort BD, Ramanathan KR, et al. Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats. Transl Psychiatry 2017;7(8):e1217.
[48] Liu H, Liu Y, Yu J, Lai M, Zhu H, Sun A, et al. Vagus nerve stimulation inhibits heroin-seeking behavior induced by heroin priming or heroin-associated cues in rats. Neurosci Lett 2011;494(1):70–4.
[49] Bouton ME, Todd TP. A fundamental role for context in instrumental learning and extinction. Behav Process 2014;104:13–9.
[50] Khoo SY, Gibson GD, Prasad AA, McNally GP. How contexts promote and prevent relapse to drug seeking. Genes Brain Behav 2017;16(1):185–204.
[51] Todd TP, Winterbauer NE, Bouton ME. Effects of the amount of acquisition and contextual generalization on the renewal of instrumental behavior after extinction. Learn Behav 2012;40(2):145–57.

[52] Childress AR, Hole AV, Ehrman RN, Robbins SJ, McLellan AT, O'Brien CP. Cue reactivity and cue reactivity interventions in drug dependence. NIDA Res Monogr 1993;137:73–95.
[53] Neisewander JL, Baker DA, Fuchs RA, Tran-Nguyen LT, Palmer A, Marshall JF. Fos protein expression and cocaine-seeking behavior in rats after exposure to a cocaine self-administration environment. J Neurosci 2000;20(2):798–805.
[54] Peters J, Kalivas PW, Quirk GJ. Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn Mem 2009;16(5):279–88.
[55] Aalbers M, Vles J, Klinkenberg S, Hoogland G, Majoie M, Rijkers K. Animal models for vagus nerve stimulation in epilepsy. Exp Neurol 2011;230(2):167–75.
[56] Ricardo JA, Koh ET. Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat. Brain Res 1978;153(1):1–26.
[57] Berntson GG, Sarter M, Cacioppo JT. Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. Behav Brain Res 1998;94(2):225–48.
[58] George MS, Sackeim HA, Rush AJ, Marangell LB, Nahas Z, Husain MM, et al. Vagus nerve stimulation: a new tool for brain research and therapy. Biol Psychiatry 2000;47(4):287–95.
[59] Semba K, Reiner PB, McGeer EG, Fibiger HC. Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. J Comp Neurol 1988;267(3):433–53.
[60] Van Bockstaele EJ, Peoples J, Telegan P. Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: evidence for a monosynaptic pathway. J Comp Neurol 1999;412(3):410–28.
[61] Dorr AE, Debonnel G. Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. J Pharmacol Exp Ther 2006;318(2):890–8.
[62] Follesa P, Biggio F, Gorini G, Caria S, Talani G, Dazzi L, et al. Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. Brain Res 2007;1179:28–34.
[63] Hassert DL, Miyashita T, Williams CL. The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. Behav Neurosci 2004;118(1):79–88.
[64] Manta S, Dong J, Debonnel G, Blier P. Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. J Psychiatry Neurosci 2009;34(4):272–80.
[65] Roosevelt RW, Smith DC, Clough RW, Jensen RA, Browning RA. Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. Brain Res 2006;1119(1):124–32.
[66] Nichols JA, Nichols AR, Smirnakis SM, Engineer ND, Kilgard MP, Atzori M. Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. Neuroscience 2011;189:207–14.
[67] Furmaga H, Shah A, Frazer A. Serotonergic and noradrenergic pathways are required for the anxiolytic-like and antidepressant-like behavioral effects of repeated vagal nerve stimulation in rats. Biol Psychiatry 2011;70(10):937–45.
[68] Chase MH, Sterman MB, Clemente CD. Cortical and subcortical patterns of response to afferent vagal stimulation. Exp Neurol 1966;16(1):36–49.
[69] Cao J, Lu KH, Powley TL, Liu Z. Vagal nerve stimulation triggers widespread responses and alters large-scale functional connectivity in the rat brain. PLoS One 2017;12(12):e0189518.
[70] Krahl SE, Clark KB. Vagus nerve stimulation for epilepsy: a review of central mechanisms. Surg Neurol Int 2012;3(Suppl 4):S255–9.
[71] Zuo Y, Smith DC, Jensen RA. Vagus nerve stimulation potentiates hippocampal LTP in freely-moving rats. Physiol Behav 2007;90(4):583–9.
[72] Lapish CC, Kroener S, Durstewitz D, Lavin A, Seamans JK. The ability of the mesocortical dopamine system to operate in distinct temporal modes. Psychopharmacology 2007;191(3):609–25.
[73] Borland MS, Engineer CT, Vrana WA, Moreno NA, Engineer ND, Vanneste S, et al. The interval between VNS-tone pairings determines the extent of cortical map plasticity. Neuroscience 2018;369:76–86.
[74] Hays SA, Khodaparast N, Ruiz A, Sloan AM, Hulsey DR, Rennaker 2nd2nd, et al. The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. Neuroreport 2014;25(9):676–82.
[75] Loerwald KW, Borland MS, Rennaker 2nd2nd, Hays SA, Kilgard MP. The interaction of pulse width and current intensity on the extent of cortical plasticity evoked by vagus nerve stimulation. Brain Stimul 2018;11(2):271–7.
[76] Rohsenow DJ, Monti PM, Rubonis AV, Gulliver SB, Colby SM, Binkoff JA, et al. Cue exposure with coping skills training and communication skills training for alcohol dependence: 6- and 12-month outcomes. Addiction 2001;96(8):1161–74.