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The lateral preoptic area and ventral pallidum embolden behavior

Rhett A. Reichard[1,2] · Kenneth P. Parsley[1] · Suriya Subramanian[1] · Hunter S. Stevenson[1] · Zachary M. Schwartz[1] · Tej Sura[1] · Daniel S. Zahm[1]

Abstract

While recently completing a study of the effects of stimulating the lateral preoptic area (LPO) and ventral pallidum (VP) on locomotion and other movements, we also noticed LPO and VP effects on motivational drive and threat tolerance. Here, we have investigated these latter effects by testing conditioned place preference (CPP), behavior on the elevated plus maze (EPM) and the willingness of sated rats to occupy a harshly lit open field center to acquire sweet pellets, a measure of threat tolerance, following infusions of vehicle or bicuculline (bic) into the LPO and VP. LPO-bic infusions robustly increased total locomotion, and, in direct proportion, occupancy of both the harshly lit field center and open arms of the EPM. LPO bic also generated CPP, but did not increase sweet pellet ingestion. These effects were attenuated by dopamine D1 and D2 receptor antagonists, whether given individually or as a cocktail and systemically or infused bilaterally into the nucleus accumbens. VP-bic infusions did not increase total locomotion, but preferentially increased field center occupancy. VP-bic-infused rats compulsively ingested sweet pellets and did so even under the spotlight, whereas harsh illumination suppressed pellet ingestion in the control groups. VP bic produced CPP and increased open arm occupancy on the EPM. These effects were attenuated by pretreatment with dopamine receptor antagonists given systemically or as bilateral infusions into the VP, except for % distance in the field center (by D1 or D2 antagonists) and pellet ingestion (by D1 antagonist). Thus, boldness generated in association with LPO activation is tightly tied to locomotor activation and, as is locomotion itself, strongly DA dependent, whereas that accompanying stimulation of the VP is independent of locomotor activation and, at least in part, DA signaling. Furthermore, respective emboldened behaviors elicited from neither LPO nor VP could clearly be attributed to goal pursuit. Rather, emboldening of behavior seems more to be a fixed action response not fundamentally different than previously for reported locomotion, pivoting, backing, gnawing, and eating elicited by basal forebrain stimulation.

Keywords Basal forebrain · Anxiety · Locomotion · Decision · Dopamine

Abbreviations

Acb	Accumbens
bic	9(R)-(−)-bicuculline methbromide
CPP	Conditioned place preference
D1	Dopamine D1 receptor
D2	Dopamine D2 receptor
EPM	Elevated plus maze
etic	S-(−)-eticlopride hydrobromide
hal	Haloperidol
i.p.	Intraperitoneal
LPO	Lateral preoptic area
SCH	R(+)-SCH-23390
VP	Ventral pallidum
VP bi	VP, infused bilaterally
VP uni	VP, infused unilaterally
VTA	Ventral tegmental area

✉ Rhett A. Reichard
reicharr@musc.edu

✉ Daniel S. Zahm
daniel.zahm@health.slu.edu

[1] Department of Pharmacology and Physiology, Saint Louis University School of Medicine, 1402 S. Grand Blvd., Saint Louis, MO 63104, USA

[2] Present Address: Department of Neuroscience, Medical University of South Carolina, 173 Ashley Avenue, Charleston, SC 29425-8908, USA

Introduction

Two distinct neural structures, the lateral preoptic area (LPO) and ventral pallidum (VP), sitting side by side in the subcommissural basal forebrain are well situated to

modulate adaptive behavior (Zahm et al. 2014a; Root et al. 2015; Barker et al. 2017; Subramanian et al. 2018). Mainly via relays in the lateral septum and accumbens (Acb), both receive dense striatal-like inputs conveying information that in some way reflects representations and recollections of the organism's internal and external environment and experiences assembled by manifold cortical and cortical-like association structures (Heimer and Wilson 1975; Swanson 1976, 2000; Heimer 1978; Beckstead 1979; Groenewegen et al. 1982, 1991; Kelley and Domesick 1982; Kelley et al. 1982; Wayner et al. 1983; Alonso and Kohler 1984; Russchen and Price 1984; Phillipson and Griffiths 1985; Caffe et al. 1987; Swanson et al. 1987a, b; McGeorge and Faull 1989; Sesack et al. 1989; Zahm 1989; Jakab and Leranth 1990; Zahm and Heimer 1990; Buchanan et al. 1994; Canteras et al. 1995; Haber et al. 1995; Chikama et al. 1997; Risold and Swanson 1997; Leranth et al. 1999; Ferry et al. 2000; Chiba et al. 2001; King et al. 2003; Vertes 2004; Reynolds and Zahm 2005; Zahm et al. 2013a; Root et al. 2015; Reichard et al. 2017). By projections to lateral hypothalamus and brainstem behavioral effectors, LPO and VP may directly influence behavioral synthesis and the autonomics (Swanson 1976; Phillipson 1979; Haber et al. 1985; Rye et al. 1987; Steininger et al. 1992; Groenewegen et al. 1993; Kalivas et al. 1993; Zahm et al. 2001; Geisler and Zahm 2005, 2006; Yetinkoff et al. 2015). By reciprocal connections with the Acb and VTA (Wayner et al. 1983; Haber et al. 1985; Zahm 1989; Groenewegen et al. 1993; Spooren et al. 1996; Zahm et al. 1996, 2013a; Yetnikoff et al. 2015; Barker et al. 2017), LPO and VP may influence the release of dopamine in the forebrain (Yokel and Wise 1975; Lyness et al. 1979; Roberts et al. 1980; Wise 1984; Abercrombie et al. 1989; Wise and Rompre 1989; Everitt 1990; Mitchell and Gratton 1996; Belujon and Grace 2015) and, via projections to the lateral habenula, forebrain levels of dopamine, serotonin, norepinephrine, acetylcholine, GABA, and other neuromodulators conveyed by long ascending neuromodulatory pathways (Sutherland 1982; Geisler and Trimble 2008; Zahm and Root 2017). By reëntrant projections relayed in the thalamus, LPO and VP may contribute to modulation of cortical representations.

Recent work in this lab has shown that the LPO and VP, despite such general similarities in connectivity, contribute in quite distinct ways to locomotion and other movements (Zahm et al. 2014a; Subramanian et al. 2018). While doing that work, we encountered evidence suggesting that the locomotor effects of stimulating the LPO and VP are accompanied by altered motivational drive and threat tolerance (Zahm et al. 2013b, 2014b). Here, we report these data formally and provide assessments of conditioned place preference (CPP) and behavior on the elevated plus maze (EPM) made following infusions of bicuculline (bic) into the LPO

and VP. Parts of the CPP and EPM data were presented at a recent conference (Reichard et al. 2016).

Materials and methods

Subjects

Male Sprague Dawley rats (250–320 g) were group and single housed prior to and following surgeries, respectively, on a 12-h light–dark cycle in a temperature- and humidity-controlled vivarium with chow and water available ad libitum. All protocols affecting them were approved by the Saint Louis University Institutional Animal Care and Use Committee in accordance with guidelines provided in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

All chemicals and drugs, except as noted, were acquired from the Sigma Chemical Company (St. Louis, MO, USA). The gamma-aminobutyric acid A ($GABA_A$) receptor antagonist, 9(R)-(-)-bicuculline methbromide (bic), and dopamine receptor antagonists, R(+)-SCH-23390 (SCH), S-(−)-eticlopride hydrobromide (etic) were dissolved in sterile 0.9% saline (Hospira Inc., Lake Forest, IL). Haloperidol (hal) was prepared in a mixture of 20% ethanol, 50% propylene glycol, and 30% distilled water as a 2 mg/ml stock solution and diluted as required with sterile 0.9% saline. Hal and etic preferentially bind D2-D3 relative to D1 class dopamine receptors, whereas SCH preferentially binds the D1 receptor (Christensen et al. 1984; Hall et al. 1985; Köhler et al. 1986; Leslie and Bennett 1987a, b; Sokoloff et al. 1990; Bourne 2001).

Guide cannulae placement

The rats were deeply anesthetized by intraperitoneal injections of a mixture of ketamine (72 mg/kg) and xylazine (11.2 mg/kg) injected as a cocktail consisting of 45% ketamine (100 mg/ml), 35% xylazine (20 mg/ml), and 20% saline at a dose of 0.16 ml/100 g of body weight. The anesthetized rats were placed into a Kopf stereotaxic instrument and incisions were made to expose the skulls, in which small burr holes were made over the LPO unilaterally and the VP, which in separate groups was targeted unilaterally (VP uni) and bilaterally (VP bi). Stainless steel guide cannulae (22 gauge) 13 mm in length were inserted to a depth 2 mm above the centers of the targets and fixed with dental cement anchored to stainless steel screws set in the skull. Stainless steel wire obturators were inserted into the guide cannulae to maintain patency. The incisions were closed with wound

clips and the rats were given i.p. injections of Buprenorphine SR (1 mg/kg) and kept warm until they awoke.

Consistent with the earlier results showing that unilateral LPO infusions of bic produce a behavioral response no different than bilateral LPO-bic infusions (Zahm et al. 2014a; Subramanian et al. 2018), only unilateral LPO-bic infusions were utilized here. In contrast, and consistent with our previous discovery of the necessity of infusing bilaterally into the VP to produce a behavioral response (see preceding refs.), the VP was infused mainly bilaterally (VP bi) in this study, except in the threat tolerance experiments, which also utilized some unilateral VP infusions (VP uni).

Behavioral testing

Three-to-five days after cannulae were implanted, the rats were brought to the behavior testing room, where they were habituated to handling on 3 consecutive days. On test days, the rats were removed from the home cages, and the obturators were removed and stainless steel injector cannulae (28 gauge) 15 mm in length, connected by a short length of polyethylene tubing (PE 20 0.38/1.09, Stoelting, Wood Dale, IL, cat. # 51155) to the Luer needle of a 1.0 µl Hamilton syringe and were inserted to a depth 2 mm beyond the tips of the guide cannulae. Infusate was propelled through the injectors at a rate of 0.25 µl/min by a syringe pump (Harvard Apparatus, Holliston, MA, model '11' plus) for a duration of 1 min, resulting in infusions of 0.25 µl. For infusions into the LPO and VP, bic was prepared at a dilution of 1 mg/3 ml, which resulted in delivery of 67 ng/0.25 µl. This concentration and rate of delivery produced robust locomotor activation in the previous studies (Reynolds et al. 2006; Zahm et al. 2014a; Subramanian et al. 2018). After the infusions, the injectors were removed and the rats were placed in one of the various test devices described below.

Locomotion and threat tolerance were measured during the rats' light period in standard activity monitors, each comprising a square, white, 43.2×43.2 cm floor and clear, 30.5 cm high plexiglass walls (Med Associates, St. Albans, VT). Fixtures containing horizontal rows of 16 point-source infrared illuminators equispaced at 2.54 cm were bracketed to the wall on one side of the chambers at heights of 2.54 and 12.7 cm from the monitor floor. Photodetectors were affixed to the opposing walls. The lower bank comprised two sets of illuminators and detectors mounted on adjacent walls at a right angle to each other so as to form a grid of 16×16 intersecting light beams for measurement of horizontal (forward) locomotion, whereas the upper consisted of a single illuminator/detector pair for detection of rearing. Beam breaks were evaluated with the aid of Activity Monitor software (Med Associates). The monitors were housed inside sound attenuating chambers with exhaust fans running (Med Associates).

To assess threat tolerance, unconditioned anxiety produced in rats by strong light and their reluctance to occupy open space were exploited. Briefly, activity monitors were modified with a ceiling mounted 90 W, 120 V, 1260 lumens spotlight aimed at the center of the chamber
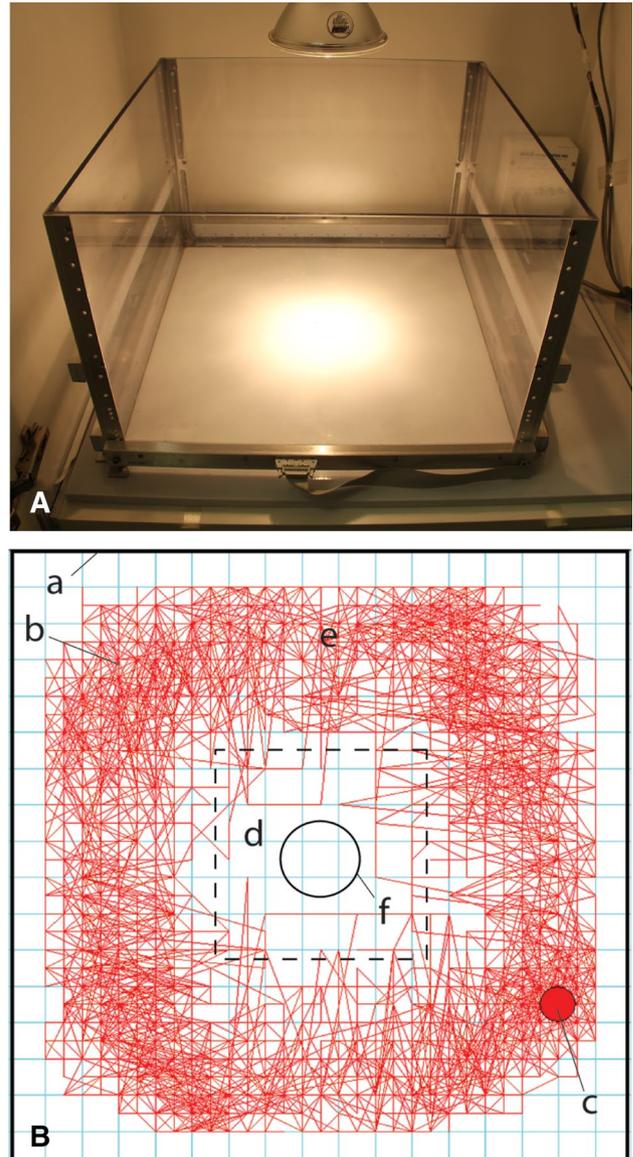

Fig. 1 Apparatus in which locomotion and threat tolerance were recorded (**A**) and a diagram of the data generated (**B**). Locomotion monitors (**A**) comprised chambers with plexiglas walls lit either by two dim incandescent bulbs situated in the upper rear corners of the sound attenuating chambers in which the monitors were enclosed or by spotlights that produced intense illumination in the center of the monitor floor, as shown. **B** provides additional detail, showing the monitor walls (a), a trace of a rat's itinerary during a test session (b), the digital representation of the rat's center of mass (c), area regarded as the field center (d, enclosed by the broken line) and periphery (e, outside of the broken line) and the diagrammatic outline of the food receptacle that was baited with sweet pellets (f)

floor (Fig. 1A) in addition to the two dim CML 182 28V 100 mA bulbs provided by the manufacturer. To determine if activations of LPO and VP affect the unconditioned tendency of rats to avoid intense illumination and the center of an open field, total distance and distance in the field center (expressed raw or as percent of total distance) were measured. Three conditions were varied—illumination (house light vs spotlight), infusate (vehicle vs bic), and the infused brain structure LPO vs VP infused unilaterally (VP uni) and bilaterally (VP bi). Because preliminary experiments (Zahm et al. 2013b, 2014b) showed that rats rarely enter a dimly or harshly lit field center, unless enticed (Fig. 1B), field centers were baited with 20 45 mg sweet pellets (TestDiet, Richmond, IN, cat. #1811256), necessitating an additional accounting of numbers of ingested pellets. To assess dopamine receptor involvement in threat tolerance, groups of rats were given subcutaneous injections of hal (0.1 mg/kg) or SCH (0.01 mg/kg) 10 min prior to being placed in the apparatus for an additional 10 min for measurement of baseline activity, after which they were removed, given intracranial infusions of bic or vehicle and returned to the apparatus for testing.

CPP was measured during the dark period using a standard CPP apparatus and dedicated software (MED-PC-IV), both acquired from Med Associates. The apparatus consists of two rectangular chambers with distinct flooring (bars vs grids) and wall colors (black with white horizontal lines vs white with black vertical lines) separated by a gray center compartment. Guillotine doors enable confinement of animals in any of the chambers. During 3 days of habituation (days 1–3), the rats were placed in the center compartment with all doors open and permitted to freely explore the apparatus for 30 min. On day 4, a pre-conditioning assessment was recorded of the time spent in each compartment followed by 3 days (days 5–7) of twice daily conditioning sessions separated by at least 1 h. Just before one of the daily conditioning sessions, bic was infused into the LPO unilaterally or VP bilaterally, after which the rats were confined in one of the large compartments (thereafter designated 'bic-paired') for 20 min. The other daily conditioning session was preceded by a vehicle infusion into the same structure and the animals were confined in the other large chamber (thereafter 'vehicle-paired') for 30 min. To offset the expectation that bic infusions elicit CPP, chambers in which animals spent less time during the pre-conditioning assessment were paired with bic infusions. The order of bic and vehicle conditioning sessions was reversed on successive days. During a post-conditioning assessment on day 8, the animals were placed in the center compartment and time spent in each chamber was recorded, as they freely explored the apparatus for 30 min. Times in the bic-paired chamber during the pre- and post-conditioning assessments were statistically compared.

EPM behavior was measured during the rats' dark period using an apparatus consisting of two 112×10 cm runways mutually bisecting at a right angle and situated 70 cm above the floor (Med Associates). One runway is open to the room and the other is enclosed. Relative time spent on the open and closed arms provides a measure of anxiety. At testing, rats received infusions of bic or vehicle into the LPO unilaterally or VP bilaterally, as described, 5 min prior to being placed at the hub, where the open and closed runways cross. Ratios of open to total arm entries, time spent on the arms after each entry and the ratio of time spent on open and closed arms were recorded. Veh and bic groups were run in counterbalanced order in successive trials to minimize repeated exposures to the apparatus.

Systemic administration of DA antagonists

To determine if dopamine receptor signaling modulates threat tolerance or motivation states elicited by LPO or VP-bic infusions, groups of animals received intraperitoneal injections of vehicle, SCH (0.01 mg/kg) or hal (0.1 mg/kg) 30 min prior to the start of all CPP conditioning sessions or 30 min before testing on the EPM.

Site-specific administration of DA antagonists—Acb

To determine if dopamine receptor signaling in the Acb is required for the expression of LPO-bic-elicited CPP, rats with implanted guide cannulae targeting the Acb bilaterally and LPO unilaterally were habituated, subjected to a pre-conditioning assessment and, just before conditioning sessions, given bilateral Acb infusions of a cocktail containing SCH (2 µg) and etic (10 µg) in 0.5 µl over 2 min and, immediately after, a unilateral infusion of bic into the LPO. Vehicle infusions into the LPO and Acb preceded confinement in the other chamber. After 3 days of such conditioning, the rats underwent post-conditioning assessment. For groups tested on the EPM, DA antagonist or veh infusions were made just prior to bic or veh infusions.

Site-specific administration of DA antagonists—VP

To determine if dopamine receptor signaling in the VP is required for VP-bic-elicited CPP, rats with guide cannulae targeting the VP bilaterally were habituated and given a pre-conditioning assessment as previously described. The rats were then given bilateral VP infusions of a cocktail containing SCH (2 µg), eticl (10 µg), and bic (67 ng) in 0.25 µl over 1 min in one chamber (bic-paired) and an equivalent volume of vehicle prior to being confined in the other chamber. After 3 days of such conditioning, a post-conditioning assessment was done. For groups tested on the EPM, DA antagonist or veh infusions were made just prior to bic or veh infusions.

Experimental groups and statistics

During the course of the study, guide cannulae were implanted, mainly bilaterally, into the LPO and VP. After a week of recovery, measurements were done on each rat with different combinations of injection (vehicle, hal, SCH) and infusion (vehicle or bic-infused unilaterally or bilaterally) on 5 consecutive days under vigilance for lethargy or other signs of illness, detection of which warranted immediate removal of a rat from the study. Duplicate measurements (same drug-infusion combination more than once in the same rat) were mostly avoided. Sample sizes are given in the legends of figures showing the data.

Threat tolerance

Three factors described above-infused structure, infusion (bic vs veh) and drug (hal or SCH vs veh)-were initially tested with a 3-way ANOVA and the Holm–Sidak post-hoc test. In view of the profound effects of the spotlight, experiments run under dim house lighting and the spotlight were subsequently tested separately using a 2-way ANOVA to distinguish structure vs infusate (veh vs bic), also assessed post-hoc with the Holm–Sidak test. The effects of hal and SCH on locomotion, % distance in the field center, and pellet ingestion were analyzed with t tests. Remaining comparisons from the threat tolerance antagonist experiments were made with an ANOVA followed by Student–Newman–Keuls post-hoc testing.

CPP

The amounts of time groups spent in the bic-paired chamber during pre-conditioning and post-conditioning assessments were statistically compared using paired t tests with the critical limit set at $P < 0.05$. Rats that spent > 70% in one chamber during the pre-conditioning assessment were excluded from analysis.

To determine the effects of dopamine antagonists, a conditioning score was calculated by subtracting time in the bic-paired chamber during the pre-conditioning assessment from time in that chamber during the post-conditioning assessment. Treatment groups were statistically compared with a one-way ANOVA and a post-hoc Holm–Sidak test with the critical limit set at $P < 0.05$.

EPM

The ratios of time spent on the open to closed arms and open to total arm entries, and the time on arms per entry following LPO and VP-bic infusions were tested statistically against the respective saline infusion values using paired t tests with the critical limit set at $P < 0.05$. A one-way ANOVA and a post-hoc Holm–Sidak test with the critical limit set at $P < 0.05$ was used to compare the effects of dopamine antagonists and vehicle.

Localization of Injections

The rats were deeply anesthetized and perfused as previously described (Zahm et al. 2014a; Subramanian et al. 2018). The brains were removed and placed in the same fixative for 4 h and then in a 30% sucrose solution overnight. Slabs of brain containing infusion sites were sectioned with a cryostat into three adjacent series of 50 μm-thick coronal sections. The first series was thaw-mounted onto subbed glass slides and the rest were stored in glass vials containing 0.1 M SPB. The mounted sections were defatted overnight in a 1:1 solution of chloroform and methanol and after rehydration in decreasing concentrations of methanol in water (100%, 95%, 90%, 80%, 70%, and 50%), placed in a filtered solution of 0.1% cresyl violet acetate in 0.3% glacial acetic acid for 10 min. The stained sections were dehydrated in increasing concentrations of MeOH and then placed in xylene prior to coverslipping with Permount. Sections were viewed with brightfield optics with an Olympus BX51 microscope and the locations of the injector tips were mapped with the aid of the Neurolucida hardware/software platform (MBF Bioscience, version 4.70.3). The maps were exported to Adobe Illustrator CS2 for preparation of illustrations.

Results

Injection sites

Infusion sites used in the threat tolerance experiments are shown in Figs. 2 and 3. For the CPP and EPM experiments, injection sites are included in the figures with the respective data. The microscopic appearance of the injection sites is comparable to that illustrated in our recent papers (Zahm et al. 2014a; Subramanian et al. 2018).

Threat tolerance

Significant main effects of illumination on total distance (3-way ANOVA, $F_{1,264} = 10.321$; $P < 0.002$) and distance in the field center (3-way ANOVA, $F_{1,264} = 23.698$; $P < 0.001$) indicated that spotlighting as compared to house lighting suppresses locomotion (note the generalized reduction of bar values across all groups in Fig. 4b, d as compared to 4a and c, respectively). Significant main effects on total distance of infused structure ($F_{1,264} = 30.681$; $P < 0.001$) and infusate ($F_{2,264} = 69.121$; $P < 0.001$) were complicated by a significant structure X infusate interaction ($F_{2,264} = 38.410$; $P < 0.001$),

Fig. 2 Diagrams showing representative unilateral infusion sites in the LPO (black dots) and VP (red dots). Additional unilateral VP infusion sites are shown in Fig. 3
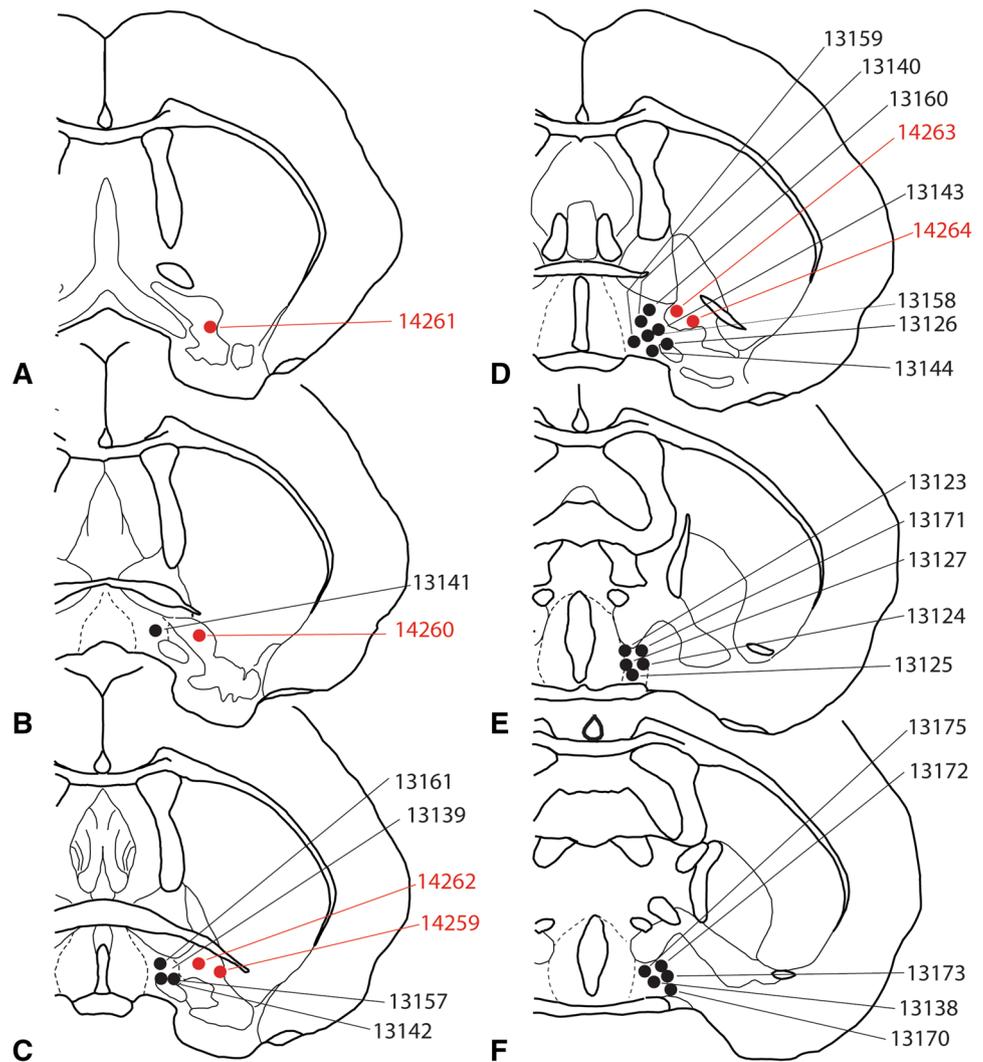
as were significant main effects on distance in the field center of infused structure ($F_{1,264} = 10.677$; $P < 0.001$) and infusate ($F_{2,264} = 45.397$; $P < 0.001$) by a structure X infusate interaction ($F_{1,264} = 15.163$; $P < 0.001$). The Holm–Sidak post-hoc test, in turn, detected multiple significant comparisons among various levels of the all of the outcomes, but no significant structure X illumination X infusate interactions. The potent effects of brilliant field center illumination on locomotion and field center occupancy suggested that separate testing of groups run under house lighting and spotlighting might better explicate the effects of bicuculline infusions. Accordingly, a description follows of the results of a 2-way ANOVA and Holm–Sidak post-hoc tests (shown on the graphs) applied to data acquired separately under dim house lighting (Fig. 4a, c, e) and spotlighting (Fig. 4b, d, f).

As we previously reported (Zahm et al. 2014a; Subramanian et al. 2018), locomotion was significantly activated by infusion of bic into the LPO ($P < 0.0001$, Holm–Sidak), but not VP uni or VP bi, whether in dim house lighting (Fig. 4a;

$F_{2,132} = 19.660$, $P < 0.001$) or under the spotlight (Fig. 4b; $F_{2,132} = 34.445$, $P < 0.001$). Expressed as relative change (Δ) after infusions (bic value minus veh value), total distance after LPO-bic infusions far outstripped that after infusions of bic into VP uni and VP bi (Fig. 4e; $F_{2,166} = 19.259$, $P < 0.001$). Pertinent to threat tolerance, infusions of bic into both the LPO and VP bi increased locomotion within the field center in both house lighting (Fig. 4c; $F_{2,132} = 6.521$, $P = 0.002$) and under the spotlight (Fig. 4d; $F_{2,132} = 9.248$, $P < 0.001$). However, Δ % distance in the center after LPO–bic infusions was strictly proportional to the increase in overall locomotion, whereas following VP bi bic infusions, Δ % distance in the field center was disproportionately increased, even (particularly) under the spot light (Fig. 4f; $F_{2,168} = 11.154$, $P < 0.001$).

Suppression of sweet pellet ingestion by the spotlight was reversed by VP infusions of bic. Testing was done shortly after a full night with ad lib access to chow, which rendered most rats sated to chow. Consequently, control rats in dim
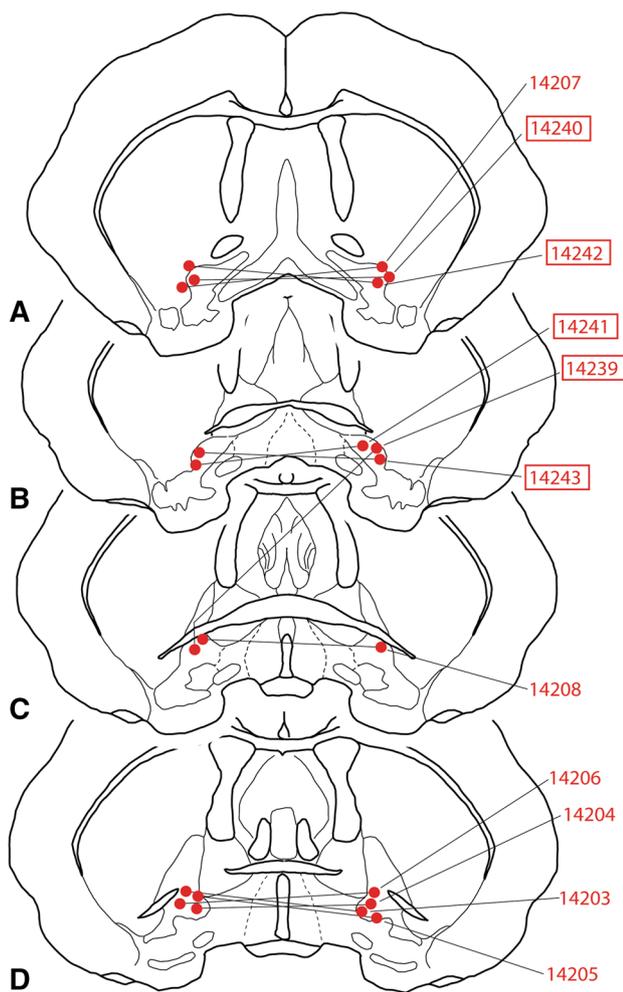
Fig. 3 Diagrams showing representative bilateral infusion sites in the VP (red dots). Those with boxed case numbers were tested, in addition, with infusions on one side only to supplement the sample of unilateral VP infusions, as shown in Fig. 2

house lighting typically ingested only half to three quarters of the 20 sweet pellets provided (Fig. 5a). The spotlight produced profound suppression of sweet pellet ingestion (Fig. 5b, as compared to 5a; 3-way ANOVA—$F_{1,264} = 52.271$; $P < 0.001$) that was partially (modestly, $P < 0.05$) and completely ($P < 0.001$) reversed, respectively, after infusions of bic into VP uni and VP bi, both in dim house lighting (2-way ANOVA—$F_{2,132} = 8.320$; $P < 0.001$) and in the spotlight (2-way ANOVA—$F_{2,132} = 15.894$; $P < 0.001$). Thus, spotlit VP bi-infused rats ingested numbers of sweet pellets similar to the house lit control groups and significantly greater than the other spotlit vehicle and bic groups ($P < 0.001$).

Following vehicle infusions into VP bi, hal given at 0.1 mg/kg profoundly suppressed ingestion of sweet pellets in house lighting and further suppressed pellet ingestion inhibited by the spotlight (Fig. 6a, $P = 0.018$ [bic vehicle,

house light]; ns [bic vehicle, spotlight]). VP bi bic infusions reversed the inhibition of sweet pellet ingestion due to spotlighting (Figs. 5b, 6a), but not that due to hal (Fig. 6a, $P < 0.001$ [bic, house light]; $P = 0.014$ [bic, spotlight]). The suppressive effect of hal on VP bi-stimulated pellet ingestion did not reflect impaired motor function, because hal did not attenuate and, indeed, may have slightly enhanced locomotion produced by VP bi bic in the spotlight (Fig. 6b). Percent distance in the field center also was increased by VP bi infusions of bic ($F_{3,18} = 15.378$, $P < 0.001$ [hal vehicle]; $F_{3,19} = 13.262$, P 0.001 [hal]), and, in contrast to sweet pellet ingestion, the increase was not attenuated by pretreatment with 0.1 mg/kg hal (Fig. 6c).

SCH given at 0.01 mg/kg also profoundly suppressed pellet taking by the vehicle-infused control group ($P = 0.005$ [bic vehicle, house light]), but not so after VP bi bic infusions (Fig. 7a). An apparent downward trend in pellet ingestion in the SCH pretreated groups following VP bi bic infusions seems likely related to the profound impairment of locomotion by SCH in the VP bi bic-infused groups (Fig. 7b, $P < 0.001$ [bic, house light]; $P = 0.002$ [bic, spotlight]). However, % distance in the field center was unaffected by SCH (Fig. 7c).

Conditioned place preference

Bic infusions into the LPO during conditioning trials (Fig. 8a–d) significantly increased the time post-conditioning spent in the bic-paired chamber (Fig. 8e) as compared to the pre-conditioning assessment (Means ± SEM: 520 s ± 30.3 vs 287s ± 33.7 [$t_{11} = -4.262$, $P = 0.001$]). This effect was blocked in rats injected before conditioning sessions with either 0.1 mg/kg hal (319 s ± 51.3 vs 364 ± 37.8; $t_{10} = 0.625$, $P = 0.55$) or 0.01 mg/kg SCH (365 s ± 42.5 vs 395.2 ± 25.7 ; $t_4 = 1.465$, $P = 0.217$). Because rats display widely varying preferences during pre-conditioning assessments, the magnitude and valence of time spent in the bic-paired chamber was expressed alternatively with the aid of a conditioning score (see "Materials and methods"). Comparison of the conditioning scores (Fig. 8f) revealed a significant main effect of drug treatment [$F_{3,36} = 7.1$, $P = 0.002$], shown by Holm–Sidak post-hoc analysis to reflect significantly lower scores after injections of 0.1 mg/kg hal ($-45 ± 71.9$) or 0.01 mg/kg SCH ($-19.5 ± 21.2$) compared to the group that received LPO-bic infusions and vehicle injections ($233 ± 54.6$). Thus, a preferred behavioral state requiring both D1 and D2 receptor signaling accompanies LPO disinhibition. Locomotion during LPO-bic-conditioning sessions (data not shown) also exhibited a significant main effect of drug treatment [$F_{3,25} = 20.1$, $P < 0.001$] revealed by Holm–Sidak post-hoc analysis to reflect significantly less locomotion in animals that received LPO-bic infusions and injections of 0.1 mg/kg hal ($1415 ± 137$) or 0.01 mg/kg
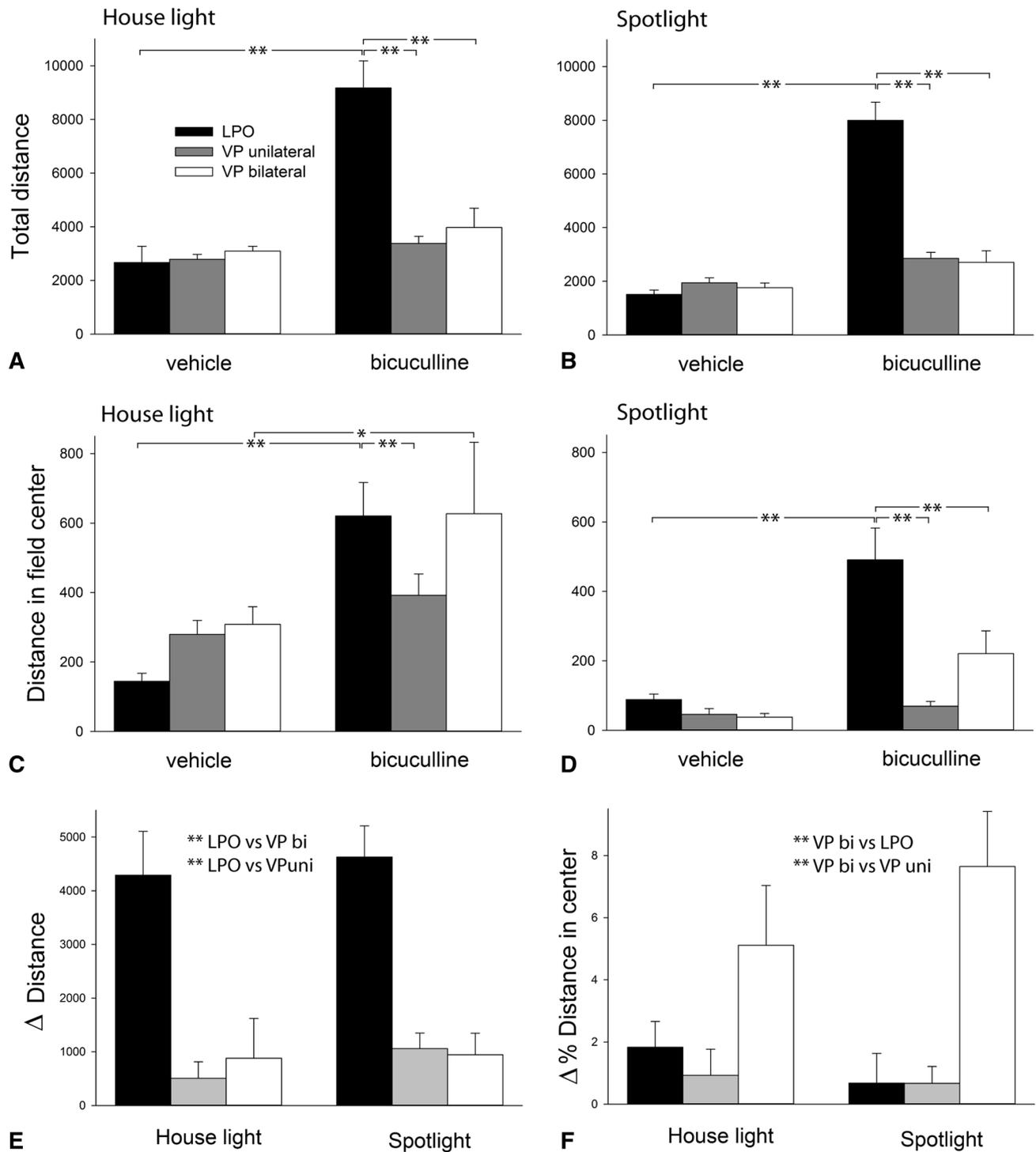
Fig. 4 Graphs showing total distance traversed (**a**, **b**) and distance traversed in the field center (**c**, **d**) after infusions of bicuculline (bic) or vehicle (veh) into the lateral preoptic area (LPO, black bars) and ventral pallidum (VP), infused either unilaterally (uni, gray bars) or bilaterally (bi, white bars), in locomotor monitors illuminated by dim house lights (**a**, **c**) or a bright spotlight aimed at the field center (**b**, **d**). **e**, **f**, Respectively, show change (Δ) in distance traversed (**e**) and Δ % of total distance traversed in the field center (**f**) due to bic infusion (calculated as bic values minus veh values). Data (n = 30 for LPO; 20 for VP uni and 19 fo VP bi) were tested with a 3-way ANOVA (results provided in the text) and, alternatively, for House light (**a**, **c**) and Spotlight (**e** and **f**), considered separately, and Δ Distance (**e**) and Δ % Distance in the center (**f**), by a 2-way ANOVA (results are given with graphs). P values: ** $0.01 < * < 0.01$
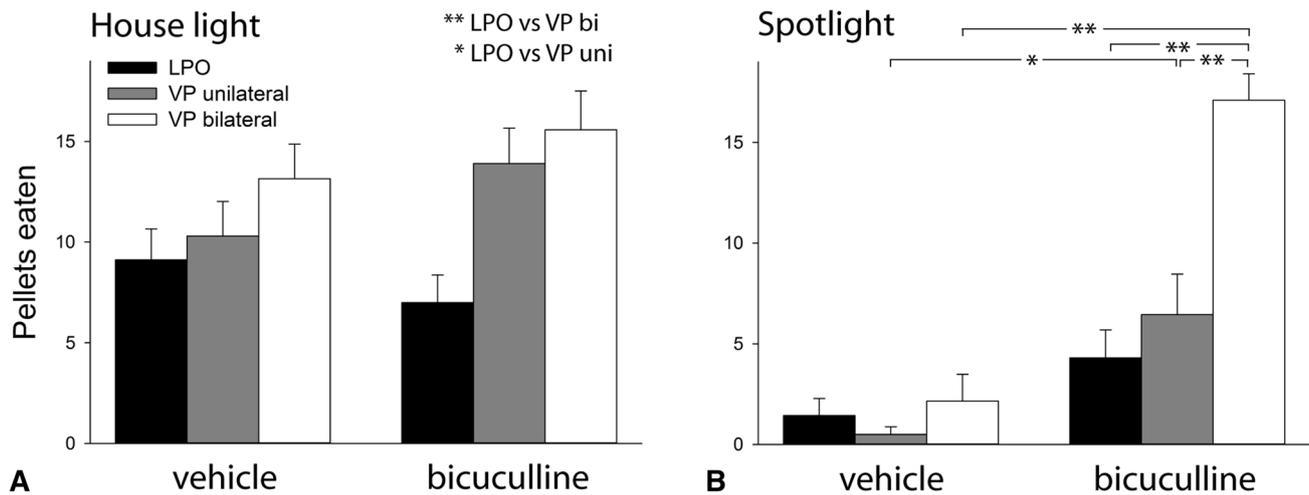
Fig. 5 Graphs showing pellets eaten after infusions of bicuculline (bic) or vehicle (veh) into the lateral preoptic area (LPO, black bars) and ventral pallidum (VP), infused either unilaterally (uni, gray bars) or bilaterally (bi, white bars), in locomotor monitors illuminated by dim house lights (**a**) or a bright spotlight aimed at the field center (**b**). Data ($n = 30$ for LPO; 20 for VP uni and 19 fo VP bi) were tested with a 3-way ANOVA (results provided in the text) and, alternatively, for House light (**a**) and Spotlight (**b**), considered separately, by a 2-way ANOVA (results are given with graphs). **$P < 0.001$; *$P < 0.01$

SCH (1479 ± 96.7) as compared to controls (2429 ± 126.9). That said, locomotion was significantly greater in all treatment groups during bic as compared to vehicle conditioning sessions.

To test if the effects of dopamine receptor antagonists on LPO-elicited CPP are due to antagonist actions in the Acb, rats with guide cannulae targeting the LPO unilaterally and Acb bilaterally underwent CPP testing. Conditioning sessions during which a cocktail containing SCH (2.0 μg) and etic (10 μg) was infused bilaterally into the Acb and bic was infused into the LPO did not result in significantly ($t_5 = 0.646$, $P = 0.547$) more time spent in the LPO-bic-paired chamber during the post-conditioning (416 ± 10.9 s) compared to the pre-conditioning (401.6 ± 12.6 s) assessment (Fig. 9a), indicating that LPO-bic-elicited CPP (Fig. 8e, f) requires dopamine receptor signaling in the Acb.

Bilateral infusions of bic into the VP during conditioning (Fig. 10a–d) significantly increased time in the bic-paired chamber (Fig. 10e) during the post-conditioning as compared to pre-conditioning assessment (538 s ± 32.8 vs 400 s ± 19.6 [$t_{11} = -4.565$, $P < 0.001$]). This effect was blocked in rats injected prior to conditioning sessions with 0.1 mg/kg hal (448 s ± 39.6 vs 424 s ± 42.6 [$t_8 = 0.009$, $P = 0.99$]) or 0.01 mg/kg SCH (401 s ± 12.6 vs 422 s ± 15.9 [$t_4 = -0.673$, $P = 0.538$]). Comparison of the conditioning scores (Fig. 10f) revealed a significant main effect of drug treatment ($F_{2,25} = 9.92$, $P = 0.003$) shown by Holm–Sidak post-hoc analysis to reflect significantly lower conditioning scores in animals that received bilateral VP-bic infusions and injections of 0.1 mg/kg hal (-0.11 ± 11.2) or 0.01 mg/kg SCH (4.6 ± 6.8) during conditioning as compared to

control groups that received bic infusions and vehicle injections (138 ± 30.3). Thus, a preferred behavioral state requiring both D1 and D2 receptor signaling accompanies VP disinhibition. Pivoting and gnawing that accompany VP disinhibition (Zahm et al. 2014a; Subramanian et al. 2018) were observed during all VP-bic-conditioning sessions.

Conditioning with bilateral VP infusions of a cocktail containing dopamine receptor antagonists and bicuculline did not significantly ($t_4 = -0.539$, $P = 0.618$) alter time in the cocktail-paired chamber during the post (389.2 ± 18.4 s) compared to the pre (385.8 ± 14.2 s) conditioning assessment (Fig. 9c), indicating that CPP elicited by VP bic (Fig. 10e, f) requires dopamine receptor signaling in the VP.

Elevated plus maze

Bic as compared to vehicle infusions into the LPO (Fig. 11a) significantly increased the ratio of time spent on the open as compared to closed arms of the EPM (0.32 ± 0.03 vs 0.09 ± 0.02 [$t_{10} = 8.56$, $P < 0.001$]). This ratio was significantly lowered [$F_{10,26} = 32.449$, $P < 0.001$] in animals pre-injected with 0.01 mg/kg SCH as compared to vehicle (0.138 ± 0.04 [$t_{16} = 3.826$, $P = 0.004$]). Unaccountably, rats that received hal at 0.1 mg/kg prior to LPO-bic infusions repeatedly fell or leapt (two subjects) from the open arms of the EPM and were not included in the statistical evaluation. Together, the results indicate that an EPM anxiolytic effect requiring signaling through D1 and D2 receptors accompanies disinhibition of the LPO.

To test if the effects of dopamine receptor antagonists on LPO-elicited EPM behavior are due to actions in the Acb,

rats with guide cannulae targeting the LPO unilaterally and Acb bilaterally underwent EPM testing. Bilateral infusions of a cocktail containing etic (10 µg) and SCH (2 µg) into the Acb significantly ($t_5=7.630$, $P<0.001$) reduced the LPO-bic-elicited open/closed arm time ratio (Fig. 9b) compared to Acb vehicle infusions (Acb cocktail + LPO bic: 0.075 ± 0.02 vs Acb saline + LPO bic: 0.386 ± 0.05). In contrast, total arm entries in this condition were not different than controls (data not shown, but see Fig. 11b). The results indicate that emboldened EPM behavior that accompanies LPO stimulation requires dopamine receptor signaling in the Acb.

Bilateral infusions into the VP of bic as compared to vehicle (Fig. 11c) significantly increased the ratio of time spent on the open as compared to closed arms of the EPM (0.674 ± 0.09 vs 0.1 ± 0.03 [$t_9=6.16$, $P=0.0001$]). This effect was restored to vehicle levels (0.1 mg/kg hal: $t_{18}=0.395$, $P=0.696$; 0.01 SCH $t_{14}=0.788$, $P=0.788$) in animals pretreated with 0.1 mg/kg hal (0.08 ± 0.02 [$F_{10,26}=32.449$, $P<0.001$]) or 0.01 mg/kg SCH (0.03 ± 0.006 [$F_{3,33}=37.9$, $P<0.001$]). Taken together, these results demonstrate that emboldened EPM behavior that accompanies bilateral disinhibition of the VP requires signaling through D1 and D2 receptors. Most rats displayed brief episodes of pivoting and gnawing in the enclosed arms and one rat was excluded from the analysis for this reason due to prolonged pivoting (180 s). Interestingly, rats never pivoted on the open arms.

Bilateral infusions into the VP of a cocktail containing bic, etic (0.1 mg/kg) and SCH (0.01 mg/kg) significantly ($t_4=10.192$, $P<0.001$) reduced the ratio of open to closed arm time (Fig. 9d) compared to bilateral VP-bic infusions (VP cocktail: 0.09 ± 0.05 vs VP bic: 0.926 ± 0.08), indicating that emboldened EPM behavior that accompanies bilateral inhibition of the VP requires D1 and D2 dopamine receptor signaling in the VP.

Comparison of LPO and VP EPM data

EPM open arm entries (Fig. 11d) significantly increased following bic as compared to vehicle infusions into the LPO (8.5 ± 0.9 vs 2.2 ± 0.7 [$t_{10}=-5.33$, $P<0.001$]) and VP (5.8 ± 0.6 vs 3.2 ± 0.6; [$t_9=-2.82$, $P=0.02$]). Significantly
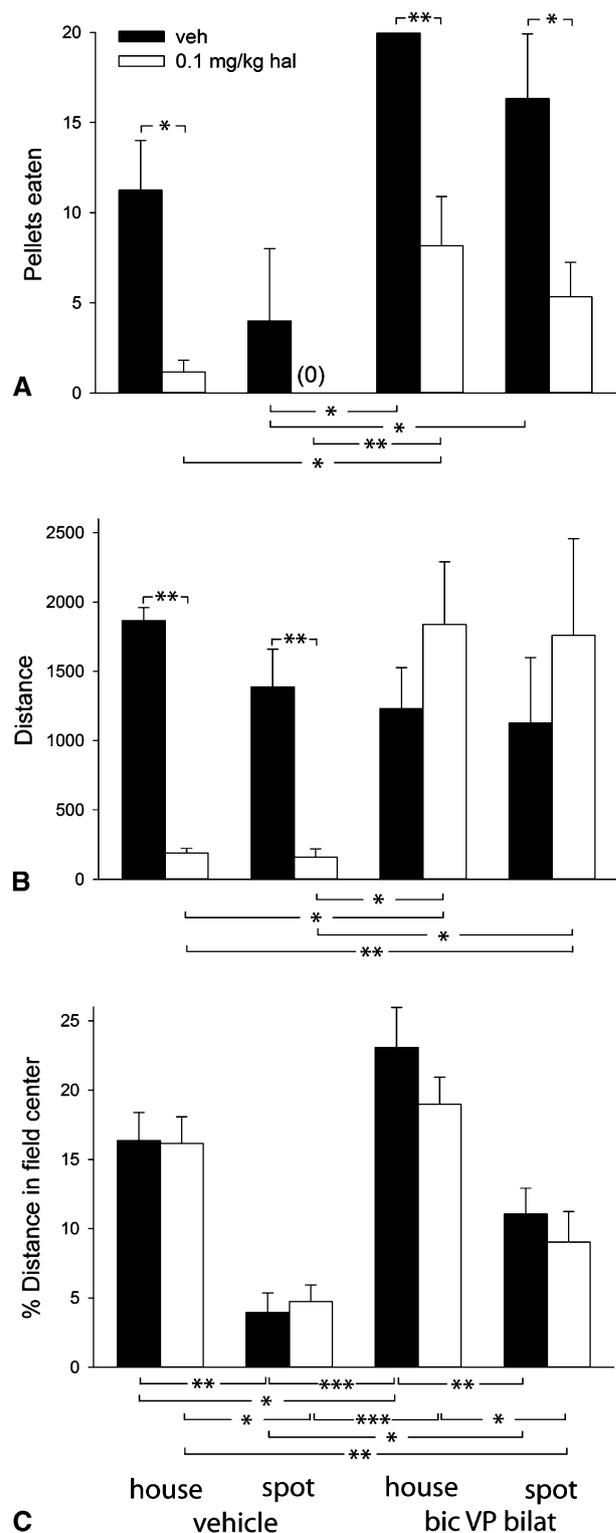
more open arm entries were recorded after LPO-bic than VP-bic infusions (Fig. 11d; [$t_{19}=2.35$, $P=0.03$]), suggesting that the emboldening effect is greater after disinhibiting the LPO than VP. However, open arm entries calculated as percent of total (Fig. 11b) were significantly more numerous
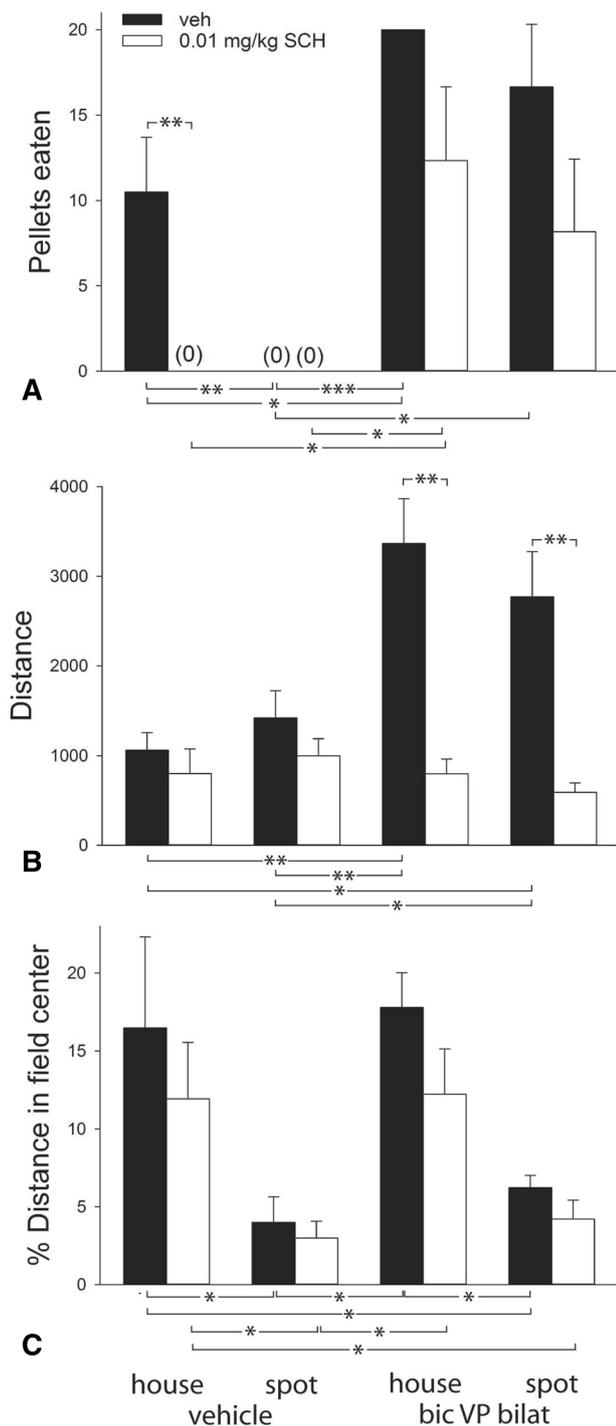
Fig. 7 Graphs showing the effects of administration of SCH-23,390 (SCH, 0.01 mg/kg) on numbers of pellets eaten (**a**), total distance traversed (**b**) and % distance of total distance traversed in the field center (**c**) following bilateral (bilat) infusions of bicuculline (bic) or vehicle (veh) into the ventral pallidum (VP) in activity monitors illuminated by dim house lights (house) or or a bright spotlight aimed at the field center (spot). Statistics are as in Fig. 6 legend

after VP than LPO-bic infusions (VP: 0.37 ± 0.02 vs LPO: 0.22 ± 0.03 [$t_{19} = -4.373$, $p = 0.02$]). Furthermore, fold increase in time on the open arms (Fig. 11e) was significantly greater following VP (5.1 ± 1.0) than LPO (2.6 ± 1.0) bic infusions ($t_{16} = 2.57$, $p = 0.02$). Accordingly, more time was spent per open arm entry (Fig. 11f) following VP bic (bic: 20.2 ± 1.4 vs sal: 7.0 ± 0.8; $t_9 = 6.96$, $p < 0.0001$) than LPO bic (bic: 7.1 ± 0.5 vs sal: 6.8 ± 1.9; $t_{19} = 9.18$, $p < 0.0001$) infusions as compared to vehicle, whereas time spent on the open arms per entry did not differ significantly between bic and vehicle infusions into the LPO. Taken together, the data indicate that, in contrast to the LPO, disinhibition of the VP produces a tolerance of EPM open arms not linked to an increase in locomotor activation.

Discussion

Based upon observed increases in % distance in the field center, sweet pellet ingestion and EPM open arm entries, summarized in Table 1, we can assert that stimulation of both the LPO and VP embolden behavior.

LPO-bic infusions produced increases in total locomotion and field center occupancy, which were directly proportional to each other, but did not increase ingestion of sweet pellets. LPO bic also increased occupancy on the open arms of the EPM, also in a manner proportionate to the potentiation of locomotion. Accordingly, the reduction in LPO-bic-elicited occupancy of the open field center and open arms of the EPM caused by SCH and hal was likely due to reductions in overall locomotion that accompanied the administration of the D1 and D2 dopamine antagonists. Indeed, the apparent emboldening effect of LPO bic was so linked to locomotion that it might alternatively be interpreted as an epiphenomenon of LPO-elicited locomotor activation. Nevertheless, LPO bic increased occupancy of space that, according to the control experiments, rats normally would prefer not to occupy—a sine qua non of emboldening. This effect might be explained, at least in part, by CPP, a reflection of increased sense of well-being that accompanied locomotion potentiated by LPO-bic infusions, in the glow of which the open field center and EPM open arms may be less threatening. To be sure, the locomotor and CPP effects of stimulating the LPO resemble those elicited by drugs that activate the mesoaccumbens DA pathway or otherwise cause release of DA in the Acb (Kelly et al. 1975), so it is consistent that LPO-bic-elicited CPP was reversed by DA antagonists infused directly into the Acb during the conditioning sessions (as well as by systemic administration of DA antagonists). Thus, a capacity of LPO stimulation to embolden behavior may occur in lock step with DA release in the Acb.
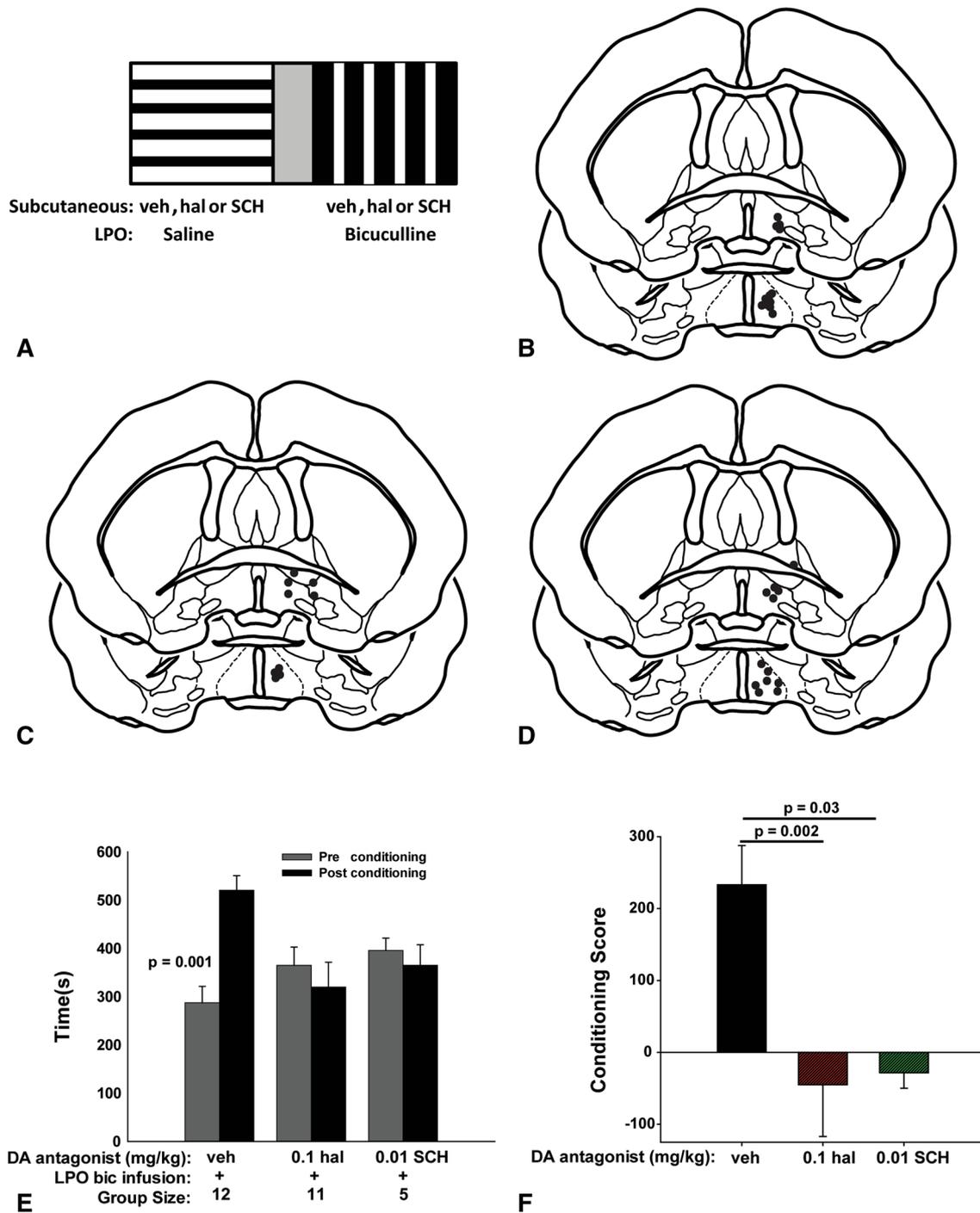
Fig. 8 Schematic illustrating LPO place preference conditioning sessions (**a**). Cannula placements for vehicle (**b**) 0.01 mg/kg SCH and 0.1 mg/kg hal (**d**) treatment groups tested in place preference and EPM. Time spent in the LPO-bic-paired chamber during the pre-conditioning (gray bars) and post-conditioning (black bars) assessments (**e**). Time in the LPO-bic-paired chamber significantly increased dur-

ing the post compared to the pre-conditioning assessment in only the control group. Statistical comparison of the conditioning scores from each treatment group shows that place preference acquired from LPO-bic infusions requires signaling through both D1 and D2 receptors (**f**)

VP-bic infusions increased field center occupancy in the absence of a general increase in locomotor activation. When field center occupancy after VP-bic infusions was calculated

as % total locomotion, it represented a significantly greater increase than after LPO-bic infusions. In addition, VP-bic-infused rats boldly ingested sweet pellets under the spotlight,
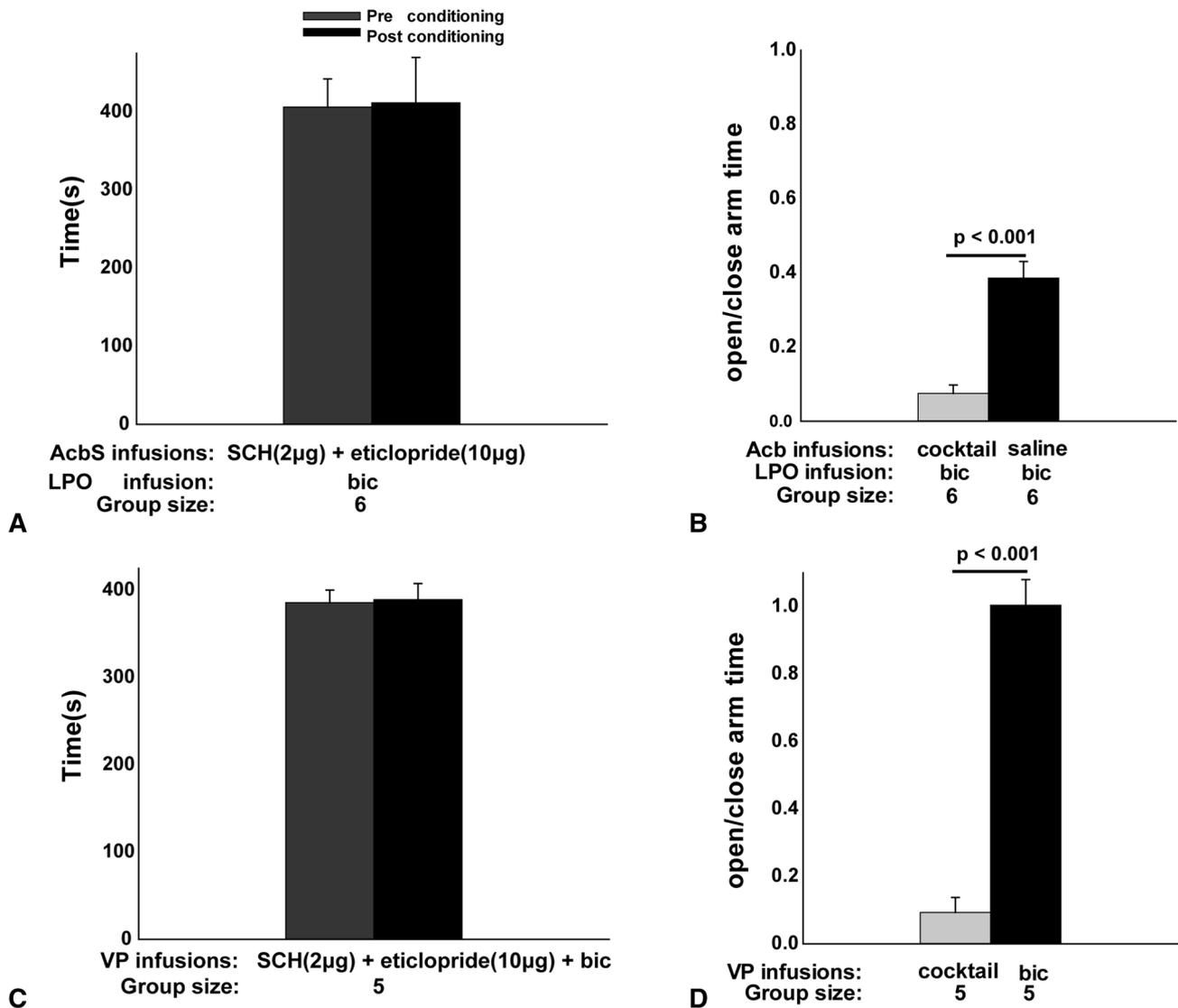
Fig. 9 Conditioning with bilateral infusions into the Acb of a cocktail containing SCH (2 μg) + eticlopride (10 μg) combined with LPO-bic infusions did not significantly alter time in the LPO-bic-paired chamber during the post- (black bar) compared to the pre- (gray bar) conditioning assessment (**a**). The ratio of open to closed arm time following LPO-bic infusions paired with bilateral Acb saline infusions (black bar) was significantly decreased when the LPO-bic infusions were paired instead with bilateral infusions into the Acb of antago-nists cocktail (gray bar) (**b**). Conditioning with bilateral infusions into the VP of a cocktail containing SCH (2 μg), eticlopride (10 μg) and bic did not significantly alter time in the cocktail-paired chamber during the post- (black bar) compared to the pre- (gray) conditioning assessment (**c**). The ratio of open to closed arm time was significantly lower following bilateral infusions into the VP of the cocktail (gray bar) as compared to infusions into VP of only bic (black bar) (**d**)

in contrast to subjects in all of the control groups, which ingested almost no pellets in harsh illumination. Presumably, pellets were ingested compulsively by the VP-bic group as a fixed behavioral response to VP-bic infusions (Cromwell and Berridge 1993; Stratford et al. 1999; Shimura et al. 2006; Smith and Berridge 2007; Smith et al. 2009; Stratford and Wirtshafter 2012, 2013; Covelo et al. 2014; Subramanian et al. 2018), rather than from hunger or to satisfy a sweet appetite. Importantly, however, VP-infused rats discounted imputed threat and aversiveness of the spotlight

to do so. Whether they were inclined to eat in the harsh light due to the strength of the compulsion, because discharging the compulsion is in itself rewarding, or because VP-bic-induced boldness promotes disregard of the spotlight was not apparent from the open field data. However, VP bic also increased open arm occupancy on the EPM, where sweet pellets were unavailable, which argues against a goal-related basis for the emboldening effect of VP. On the other hand, VP-bic-elicited pellet ingestion was attenuated by the DA D2 receptor antagonist hal, which renders
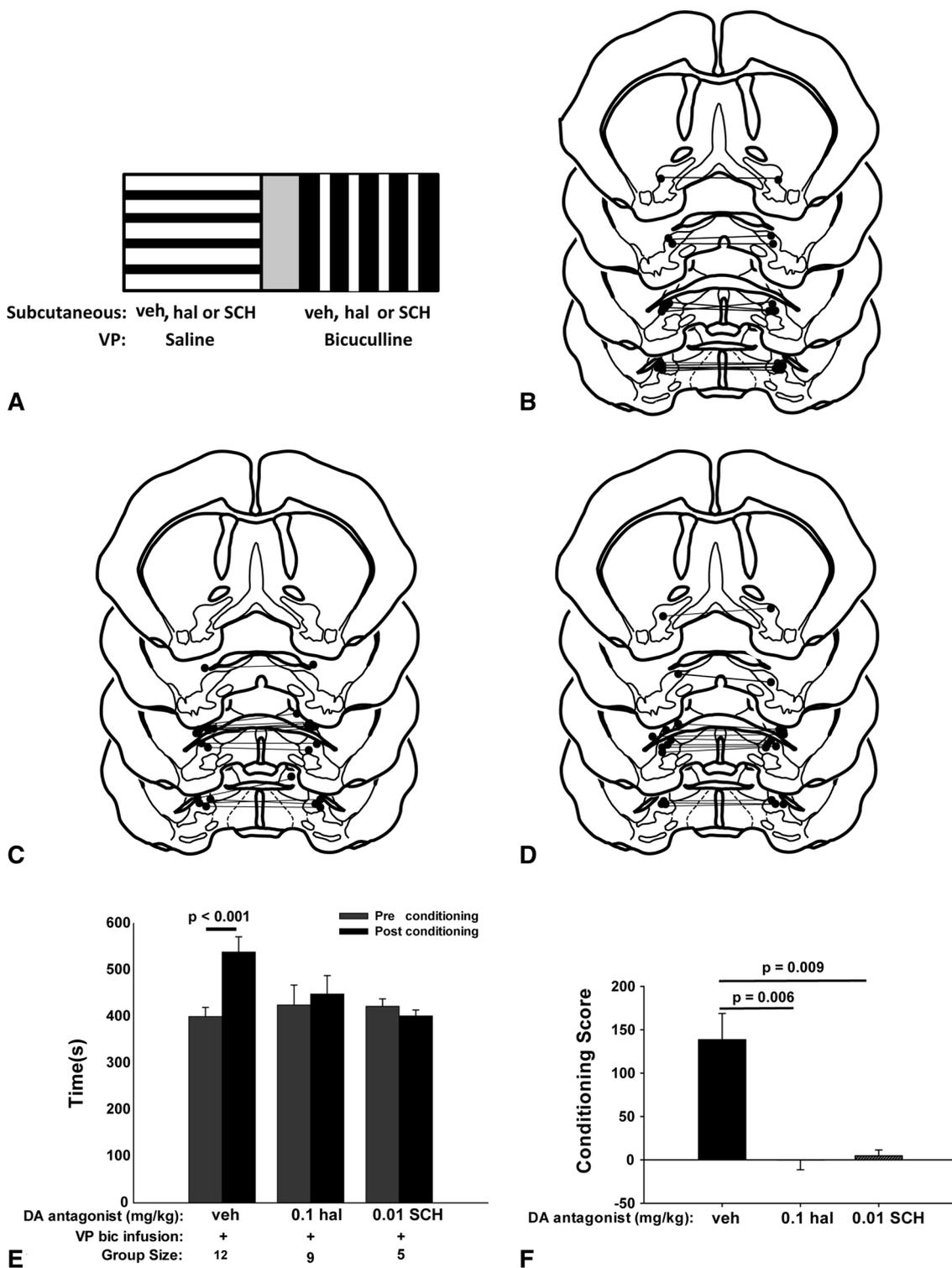
Fig. 10 Schematic illustrating VP place preference conditioning sessions (**a**). Cannula placements for vehicle (**b**) 0.01 mg/kg SCH (**c**) and 0.1 mg/kg hal (**d**) treatment groups tested in CPP and EPM. Time spent in the VP-bic-paired chamber during the pre-conditioning (gray bars) and post-conditioning (black bars) assessments (**e**). Time in the VP-bic-paired chamber significantly increased during the post compared to the pre-conditioning assessment in only the control group. Statistical comparison of the conditioning scores from each treatment group showed that place preference acquired from VP–bic infusions requires signaling through both D1 and D2 receptors (**f**)

reinforcers less reinforcing (Wise 2004). In contrast to pellet ingestion, center field occupancy increased by VP-bic infusions remained increased in the presence of hal. Inasmuch as infusions of bic into the VP also produced CPP, enhanced sense of well-being may underlie the willingness of VP-bic-infused rats to occupy (and ingest pellets in) the harshly lit field center. Were this the case, however, a treatment that prevents VP-bic-associated development of CPP would be expected also to block the VP-bic-elicited increase in field center occupancy. As it turned out, hal prevented VP-bic-generated CPP, suggesting that the failure of hal to decrease VP-bic-elicited field center occupancy may more reflect a sort of threat blindness not associated with well-being. These discrepancies are at present unexplained, but it is pertinent that Floresco et al. (2003) reported that stimulation of VP does not activate ventral tegmental DA neurons or cause release of DA in the Acb, which suggests, in any event, that the behavioral effects of VP stimulation are independent of DA release in the Acb (see also below). However, our data suggest that VP-bic-elicited pellet ingestion, at least, requires D2 receptor stimulation somewhere in the brain, possibly the VP itself, insofar as we also show that selective blockade of D2 + D1 receptors in the VP blocks VP-bic-elicited CPP and EPM open arm occupancy. Obviously, these interactions require further investigation, but what is not in dispute is that rats subjected to VP-bic infusions reliably and vigorously ingest sweet pellets in a harshly illuminated field center and boldly occupy the open arms of the EPM, whereas control rats do not.

We thus have abundant evidence linking of one sort of emboldened behavior to stimulation of the LPO and another to activation of the VP. Boldness generated in association with LPO activation is tightly tied to locomotor activation and, as is locomotion itself, strongly DA-dependent (Kelly et al. 1975), whereas that accompanying stimulation of the VP was independent of locomotor activation and, at least as regards field center occupancy in harsh illumination, DA signaling. Furthermore, in the case of neither LPO nor VP could the respective emboldened behavior be clearly attributed to goal pursuit—sweet pellet ingestion was not increased after LPO stimulation and, after VP activation, pellet consumption is a fixed action response unrelated to normal feeding cues. Rather, the data suggest that emboldening of behavior is itself an obligatory response linked to the downstream effects of stimulating the VP much akin to other fixed action motor responses, such as pivoting, backing, gnawing and eating elicited by VP stimulation (Subramanian et al. 2018).

Methodological considerations

This paper is the fourth in a series from our lab describing behavioral effects of bic infusions into the LPO and VP.

Important methodological concerns, such as the localization and appearance of infusion sites, control infusions and mechanism of action of bic have been addressed at length in the earlier papers, which were concerned exclusively with motor responses (Reynolds et al. 2006; Zahm et al. 2014a; Subramanian et al. 2018).

CPP

Notably, our assessment of VP-bic-elicited CPP differs from Gong et al. (1997), who reported no CPP after VP bic. This discrepancy may reflect differences in cannula placements or numbers of VP-bic conditioning sessions (two in Gong et al., three here). Furthermore, the CPP and EPM experiments in this study were performed during the dark part of the diurnal cycle, when rats are active. Two groups of our rats ($n = 12$) did not show CPP after conditioning during light hours and actually spent very little time exploring the apparatus during the post-conditioning assessment (data not shown), consistent with the negative result of Gong et al. (1997).

Concentrations of Hal and SCH

Relatively low doses of hal at 0.1 mg/kg and SCH at 0.01 mg/kg strongly suppressed basal locomotion and produced moderate attenuation of forebrain-stimulated locomotion, which raises the possibility that accompanying reductions of field center occupancy and pellet ingestion reflected impairments of performance rather than motivation. This was addressed by normalizing critical data, such as field center distance and open arm occupancy as % of total. In contrast, the effects of DA antagonists on pellet ingestion were less susceptible to motor impairments. First, insofar as VP bic rescues the hal-elicited motor deficit (Subramanian et al. 2018; present data), the accompanying hal-elicited reduction in VP-bic-elicited pellet ingestion, CPP and EPM open arm occupancy cannot be attributed to motor impairment. Alternatively, pellet ingestion was not significantly reduced by SCH after VP-bic infusions, despite a moderate to severe impairment in locomotion, which is consistent with a minimal effect of the D1 antagonist and, for that matter, motor impairment, on VP-bic-elicited eating. Preliminary experiments with lower doses of hal and SCH that left locomotion mildly impaired, had no effects on the critical dependent variables of the study, i.e., % distance in the field center, pellet consumption, CPP and EPM. Alternatively, increasing the concentrations of hal and SCH beyond 0.1 and 0.01 mg/kg, respectively, produced pronounced suppression of locomotion, which prevented assessment of behavior, i.e., rats remained stationary in a corner of the apparatus throughout the trials.

A

B

C

D

E

F
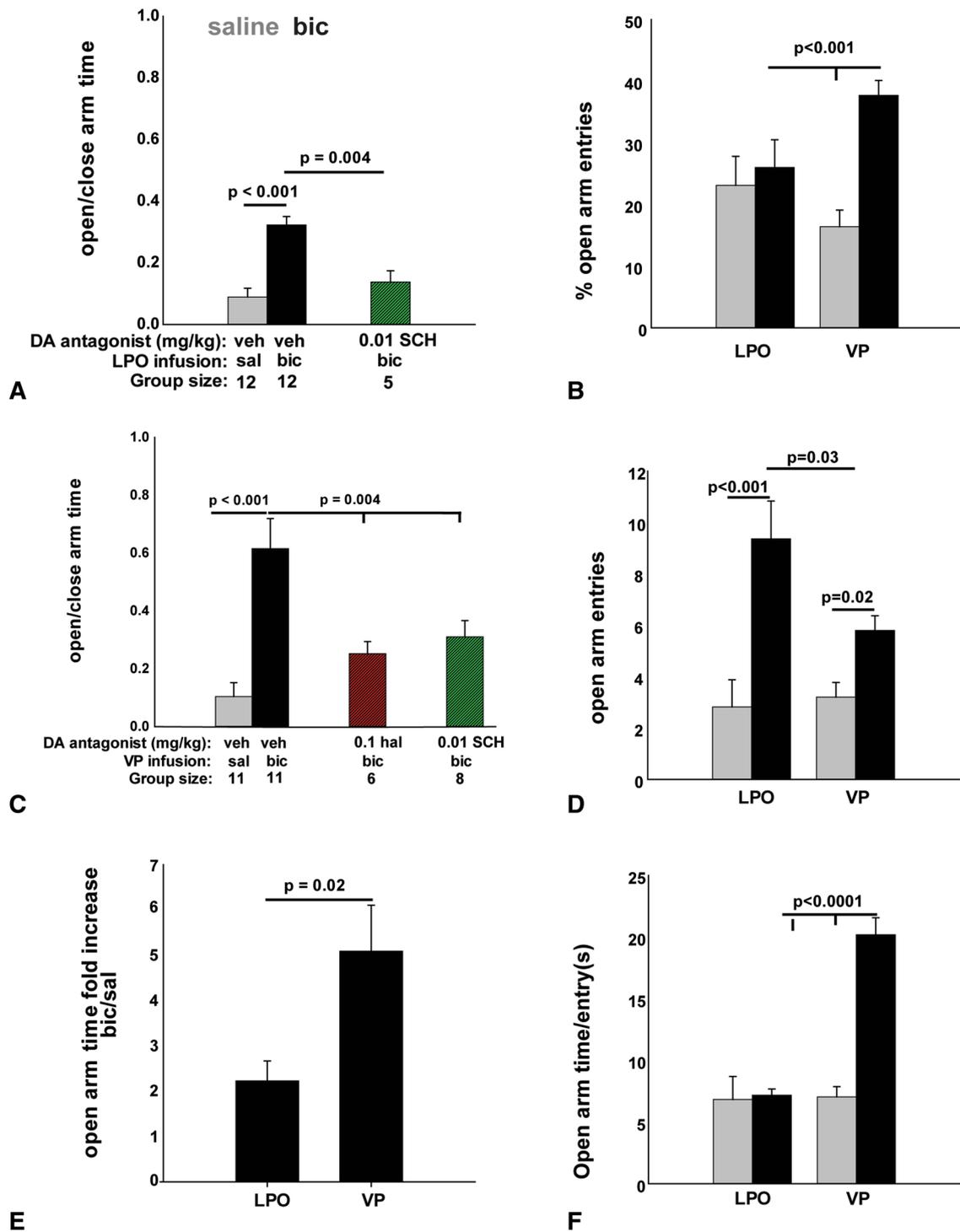
Effects of systemic and site-specific DA antagonists

Our data showed that alterations in CPP and EPM behavior elicited by disinhibition of the LPO require DA receptor signaling in the Acb, but did not exclude the possibility that DA receptor stimulation is also required elsewhere, e.g., in the VP. Alternatively, by co-infusing bic and DA antagonists into the VP we showed that VP-elicited behavioral states require DA receptor signaling in the VP, but, again, this did not exclude a possibility that DA receptor stimulation is also required elsewhere, e.g., in the Acb. Despite our keen interest to explore these alternatives more fully, technical constraints prevented us from infusing bic into the LPO along with DA antagonists bilaterally into the VP or bic

◄**Fig. 11** **a** The ratio of open to closed arm time significantly increased following LPO-bic infusions (black bar) as compared to LPO saline infusions (gray bar). The bic-elicited increase was attenuated by pretreatment with SCH (green hashed bar). However, VP-bic infusions elicited a higher percentage of open arm entries, expressed as percent of total, compared to VP saline or LPO-bic infusions which were not significantly different than LPO saline (**b**). **c** The ratio of open to closed arm time significantly increased following VP-bic infusions (black bar) compared to VP saline infusions (gray bar). The bic-elicited increase was significantly attenuated by pretreatment with haloperidol (red hashed bar) or SCH (green hashed bar). The number of open arm entries was significantly increased by bic infusions (black bars) into the LPO or VP as compared to the respective saline infusions (gray bars). **d** Open arm entries were significantly more numerous following bic infusions into the LPO compared to VP. **e** The fold increase in time spent on the plus maze open arms following bic as compared to saline was significantly greater for VP as compared to LPO infusions. **f** VP-bic infusions increased the time spent each time the animal entered an open arm as compared to VP saline or LPO-bic infusions. Open arm time spent per entry was no different following LPO bic as compared to LPO saline infusions

bilaterally into the VP along with DA antagonists bilaterally into the Acb. Fortunately, the literature contains much information useful to the interpretation of the experiments we were able to do.

Inasmuch as the LPO and VP both project similarly to brainstem behavior effectors and structures that influence them (refs. cited in the "Introduction"), both LPO and VP should be able to influence motivated behaviors independently of DA release in the Acb. However, this seems unlikely to be the case for LPO-bic infusions, because co-infusing DA antagonists into the Acb prevented the increases in CPP and EPM open arm tolerance. That is, if LPO disinhibition sends motivationally relevant signals downstream, independent of Acb DA release, CPP and emboldened EPM open arm behavior should not be affected by blockade of DA receptors upstream in the Acb. Alternatively, behavioral boldness elicited by VP-bic infusions was disrupted by DA antagonists co-infused within the VP itself, which was not unexpected given that VP activation does not increase the activity of VTA DA neurons or Acb DA release (Floresco et al. 2003). In point of fact, stimulation and inhibition of VP or VP terminals in the VTA, respectively, suppressed and increased the firing rates of VTA dopamine neurons (Wu et al. 1996; Floresco et al. 2003; Hjelmstad et al. 2013; Mahler et al. 2014). Accordingly, it seems unlikely the CPP response to VP bic is contingent upon dopamine signaling in the Acb. Instead, CPP elicited by bic infusions into the VP functionally resembles CPP due to VP psychostimulant infusions—both act to increase extracellular DA concentration in the VP (Gong et al. 1996, 1998)—consistent with reports that, e.g., stimulation of VP DA receptors elicits conditioned reward (Fletcher et al. 1998) and facilitates cocaine CPP (Gong et al. 1997) and cocaine self-administration (Sizemore et al. 1998). Furthermore, VP lesions

and inactivation of VP block the rewarding effects of Acb DA (Swerdlow et al. 1983) and drugs that increase Acb DA concentrations (Hubner and Koob 1990; Hiroi and White 1991; Subramanian et al. 2018). Thus, motivational potency typically attributed to Acb dopamine release may actually be conveyed to behavioral effectors, at least in part, by outputs from the VP. If neural substrates for motoric (Sherrington 1910; Brown 1911; Woods 1964; Chaurand et al. 1974; Micco 1974; Waldbillig 1975) and motivational (Bard 1928, 1934a, b; Wolfle et al. 1971; Glickman and Schiff 1967; Venkatraman et al. 2017) responding are intrinsic to the brainstem and disinhibition of the VP elicits both motoric and motivational responding, it is reasonable to speculate that VP conveys a motivationally relevant signal directly to the brainstem and/or, via other ascending modulatory projections and the thalamus, to the telencephalon (Zahm 2006; Heimer et al. 2008; Zahm and Root 2017), independently of Acb DA release. However, just as VP-bic infusions fail to generate the kind of (integrated, coordinated) locomotor activation that accompanies LPO stimulation or DA release in the Acb (Subramanian et al. 2018), the 'emboldening' response to VP bic also lacks a genuine, i.e., goal-linked, motivational component, and thus may be better regarded as another "fixed action" response along with pivoting dyskinesias, compulsive gnawing and so on, observed after activation of the VP (Subramanian et al. 2018).

Concluding remarks

A consistent theme that over the years has characterized conceptualizations of the neural substrates of adaptive behavior is that cognitive-affective states proceed from telencephalic integration of neural signaling reflecting genetically transmitted behavioral imperatives and the external and internal environments via pathways that descend to motor networks—behavioral programs—intrinsic to the brainstem and spinal cord (Sherrington 1910; Brown 1911; Bard 1934a, b; Chaurand et al. 1974; Micco 1974; Waldbillig 1975; Woods 1964; Nauta 1986; Alheid and Heimer 1988; Swanson 1987, 2000; Nieuwenhuys 1996; Zahm 2000, 2006; Pinker 2003; Morgane et al. 2005; Heimer et al. 2008; Uddin et al. 2014; Barrett 2017; Pessoa 2017). A crucial advance in this progression owes to Lennart Heimer, George Alheid, Jose de Olmos and other of their colleagues in the conceptualization of basal forebrain functional–anatomical macrosystems (e.g., Heimer 1972; Heimer and Wilson 1975; Alheid and Heimer 1988; Heimer and Alheid 1991; Heimer et al. 1991, 2008; McDonald 1991; McDonald et al. 1996, 1999; Shi and Cassell 1998; Zahm 1999, 2000; Shammah-Lagnado and Santiago 1999, 2006; Reynolds and Zahm 2005; Heimer and Van Hoesen 2006; Reichard et al. 2017). It is implicit in the organization of macrosystems that competing and

Table 1 Summary of experiments and results

Treatments			Effects[a]				
			Threat tolerance experiments			CPP	EPM open arms
Bicuculline	Dopamine antagonists		Total distance	% distance in center	Pellets eaten		
Site of infusion	Specificity	Site of delivery					
LPO			↑	↔	↔	↑	↑
	D2	Systemic	Block	n/a	n/a	Block	Block[b]
	D1	Systemic	Block	n/a	n/a	Block	Block
	D2+D1	Acb bi	nd	n/a	n/a	Block	Block
VP bi			↔	↑	↑	↑	↑
	D2	Systemic	n/a	↔	Block	Block	Block
	D1	Systemic	n/a	↔	↔	Block	Block
	D2+D1	VP bi	n/a	nd	nd	Block	Block

Acb bi accumbens, bilateral, *CPP* conditioned place preference, *D1* dopamine D1 receptor, *D2* dopamine D2 receptor, *EPM* elevated plus maze, *LPO* lateral preoptic area, *VP bi* ventral pallidum, bilateral, ↑ increased or produced, ↔ no effect, *block* effect reversal, *n/a* - not applicable, *nd* not done

[a] Symbols show the effects of LPO and VP bi bicuculline infusions on the indicated experimental parameters and of dopamine antagonists on effects elicited by LPO- and VP bicuculline

[b] Rats jumped off the open arms (see "Results")

cooperating pathways descend to modulate adaptive movements and motivational tone by acting on influencers of brainstem behavioral effectors and long reëntrant ascending and thalamocortical pathways. The present study aligns with this model of brain organization by showing that two output structures, LPO and VP, serving adjacent basal forebrain macrosystems act by different mechanisms to embolden behavior.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Human Participants and/or Animals There were no human subjects. Male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 250–375 g were used in accordance with policy mandated in the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (http://grants.nih.gov/grants/olaw/references/phspol.htm) provided by the U.S. Department of Health and Human Services with oversight by the Saint Louis University Animal Care Committee. Veterinary care was provided by the Saint Louis University Department of Comparative Medicine.

Ethical approval All authors read and approved the final submitted manuscript.

Informed consent There were no human subjects.

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