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Dopaminergic neurotransmission in ventral and dorsal striatum differentially modulates alcohol reinforcement

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Abstract

Dopaminergic neurotransmission in the striatum has been widely implicated in the reinforcing properties of substances of abuse. However, the striatum is functionally heterogeneous, and previous work has mostly focused on psychostimulant drugs. Therefore, we investigated how dopamine within striatal subregions modulates alcohol-directed behaviour in rats. We assessed the effects of infusion of the dopamine receptor antagonist alpha-flupenthixol into the shell and core of the nucleus accumbens (NAcc) and the dorsolateral striatum (DLS) on responding for alcohol under fixed ratio 1 (FR1) and progressive ratio (PR) schedules of reinforcement. Bilateral infusion of alpha-flupenthixol into the NAcc shell reduced responding for alcohol under both the FR1 (15 μg/side) and the PR schedule (3.75–15 μg/side) of reinforcement. Infusion of alpha-flupenthixol into the NAcc core (7.5–15 μg/side) also decreased responding for alcohol under both schedules. By contrast, alpha-flupenthixol infusion into the DLS did not affect FR1 responding, but reduced responding under the PR schedule (15 μg/side). The decreases in responding were related to earlier termination of responding during the session, whereas the onset and rate of responding remained largely unaffected. Together, these data suggest that dopamine in the NAcc shell is involved in the incentive motivation for alcohol, whereas DLS dopamine comes into play when obtaining alcohol requires high levels of effort. In contrast, NAcc core dopamine appears to play a more general role in alcohol reinforcement. In conclusion, dopaminergic neurotransmission acts in concert in subregions of the striatum to modulate different aspects of alcohol-directed behaviour.

Introduction

Alcohol use disorder (AUD) is a chronic relapsing brain disorder characterized by excessive and compulsive alcohol use that affects approximately 76 million people worldwide (WHO, 2011; American Psychiatric Association, 2013). In order to develop more effective treatments for AUD, the neural mechanisms of this disorder have been intensively investigated during the last decades (Spanagel, 2009; Barker & Taylor, 2014). In this regard, the dopaminergic innervation of the striatum belongs to the most widely investigated neural systems involved in addictive behaviour, including AUD (Robinson & Berridge, 1993; Gonzales *et al.*, 2004; Wise, 2004; Everitt & Robbins, 2005; Spanagel, 2009; Koob & Volkow, 2010; Luscher & Malenka, 2011; Salamone & Correa, 2012).

The striatum is an anatomically and functionally heterogeneous structure (Zahm, 1999; Voorn *et al.*, 2004; Everitt & Robbins, 2005; Yin *et al.*, 2008; Balleine & O'Doherty, 2010; Sesack & Grace, 2010; Floresco, 2015). The midbrain dopaminergic innervation of the striatum is organized in a mediolateral fashion, whereby medial regions of the ventral tegmental area (VTA) project to the nucleus accumbens (NAcc) shell, and more lateral parts of the VTA project to the NAcc core. The substantia nigra pars compacta, which lies dorsolateral from the VTA, innervates the dorsal striatum (Björklund & Dunnett, 2007; Ikemoto, 2007). The striatum also receives topographically organized glutamatergic inputs from the frontal cortex, amygdala, thalamus and hippocampus (Zahm, 1999; Voorn *et al.*, 2004; Sesack & Grace, 2010). Broadly speaking, these inputs provide information related to higher cognitive functions (frontal cortex), learned associations and emotions (basolateral amygdala), context (hippocampus) and arousal (medial thalamus), the processing of which is modulated by dopaminergic inputs to the striatum (Voorn *et al.*, 2004; Everitt & Robbins, 2005; Sesack & Grace, 2010; Floresco, 2015). As a result, the selection and generation of actions on the basis of emotionally coloured information relies on coordinated striatal function, whereby different subregions mediate distinct aspects of this function. That is, the NAcc core has been implicated in cue-controlled behaviour, the NAcc shell in behavioural vigour and the maintenance of behavioural focus, whereas

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the dorsolateral striatum (DLS) is best known for its role in stimulus-response habits (Voorn *et al.*, 2004; Everitt & Robbins, 2005; Yin *et al.*, 2008; Balleine & O'Doherty, 2010; Floresco, 2015).

Consistent with its functional heterogeneity, findings from neurochemical and electrophysiological studies suggest regional specificity in the effects of alcohol in the striatum (Chen *et al.*, 2011; Adermark *et al.*, 2013; DePoy *et al.*, 2013; Fanelli *et al.*, 2013; Logrip *et al.*, 2015). Indeed, the NAcc core has been implicated in cue-controlled alcohol seeking (Chaudhri *et al.*, 2008, 2010; Gremel & Cunningham, 2008), while the NAcc shell is thought to contribute to the primary reinforcing properties of alcohol (Howard *et al.*, 2008; Engleman *et al.*, 2009; Ding *et al.*, 2015) and to context-induced alcohol seeking (Chaudhri *et al.*, 2009; Hauser *et al.*, 2015). By contrast, the DLS has been shown to be involved in habitual alcohol seeking (Corbit *et al.*, 2012, 2014). Together, these studies suggest a differential involvement of ventral and dorsal striatal subregions in responding for alcohol. However, it is unknown how dopamine within these striatal subregions modulates alcohol-reinforced behaviour.

In this study, we therefore used infusions of the dopamine receptor antagonist alpha-flupenthixol to systematically assess the role of dopamine in different striatal subregions in responding for alcohol using a fixed ratio 1 (FR1) and a progressive ratio (PR) schedule of reinforcement in rats. Considering that different roles for the NAcc shell and core in alcohol reinforcement have been reported (see above), we hypothesized that alpha-flupenthixol infusions into the NAcc would alter responding for alcohol in a subregion and reinforcement schedule-dependent manner. With respect to the DLS, recent studies have shown that dopaminergic signalling in this brain region might, alongside its role in drug-seeking habits, also be involved in the reinforcing properties of cocaine (Veeneman *et al.*, 2012, 2015; Willuhn *et al.*, 2012). Therefore, we hypothesized that dopamine receptor blockade in the DLS would affect alcohol self-administration under both FR and PR schedules of reinforcement.

Materials and methods

Animals

Male Lister Hooded rats (Charles River, Sulzfeld, Germany) weighing 220–250 g upon arrival in the laboratory were housed individually under controlled temperature and humidity conditions on a 12-h reversed light-dark cycle (lights off at 7.00 AM) with *ad libitum* access to water and chow. Rats were allowed 2 weeks of acclimatization to the housing conditions before experiments commenced. They were handled and weighed at least once a week throughout the experiment. All experiments were approved by the Animal Ethics Committee of Utrecht University and conducted in agreement with Dutch laws (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Intermittent alcohol access in the home cage

The rats were provided with intermittent access to alcohol (20% v/v) and water in a two-bottle choice setup in the home cage for a period of 2 months. The rats were exposed to alcohol for 3 days a week (Monday–Wednesday–Friday) for 7 h between 9.00 AM and 16.00 PM (i.e., during the active phase of the animals) in the first month and access was extended to 24 h/session in the second month. Bottles were weighed before and after each session, and the placement of the alcohol bottle was alternated between sessions to avoid the development of a side preference. We have observed marked individual differences in alcohol intake and preference between Lister Hooded rats. Therefore, after 2-month intermittent alcohol access (IAA), the rats were ranked based on the animals' average alcohol intake per week and were assigned ranking scores (Spoelder *et al.*, 2015a). Rats within the lower and upper 25% of the total ranking score range were designated as low and high alcohol drinking rats, respectively, and were used for other studies. The middle 50% were used in this study, so that experimental groups with relatively little variability in alcohol reinforcement could be used (Spoelder *et al.*, 2015a). These rats were assigned to one of three groups to be implanted with cannulas aimed at NAcc shell, NAcc core or DLS, taking their average alcohol intake into account, to ensure similar levels of alcohol intake between groups before operant alcohol self-administration commenced.

Surgery

One week after cessation of IAA, the rats were implanted with bilateral 26-gauge guide cannulas (Plastic One, Roanoke, VA, USA) targeting the NAcc shell (anteroposterior (AP) + 1.4 mm; mediolateral (ML) ± 2.0 mm; dorsoventral (DV) −6.8 mm ventral at an angle of 5°), the NAcc core (AP + 1.4 mm; ML ± 2.0 mm; DV −5.8 mm at an angle of 5°) or the DLS (AP + 0.8 mm; ML ± 3.4 mm; DV −3.3 mm) with coordinates relative to bregma (Paxinos & Watson, 2004). The guide cannula was aimed at 2.0 mm above the target region. Cannulas were fixed to the skull using stainless steel screws and antibiotic cement (Simplex™ P bone cement with tobramycin, Stryker Nederland B.V., The Netherlands). Anaesthesia and analgesia protocols were as previously published (Schaap *et al.*, 2012, 2013). Briefly, rats were anaesthetized with fentanyl (0.25 mg/kg, IP – Fentanyl Janssen®; Janssen-Cilag B.V., The Netherlands) and dexmedetomidine (0.15 mg/kg, IP – Dexdomitor®; Pfizer Animal Health B.V., The Netherlands) in their home cage. After loss of the pedal reflex, the animals were transported to the surgery room and, after endotracheal intubation, anaesthesia was maintained with isoflurane if necessary. Upon completion of the surgery, anaesthesia was terminated with atipamezole (0.6 mg/kg, IP – Antisedan®; Pfizer Animal Health B.V., The Netherlands), and the animals received buprenorphine (0.05 mg/kg, IP – Buprecare®; AST Farma B.V., The Netherlands) for pain relief. For postoperative analgesia, the rats were treated with buprenorphine (0.05 mg/kg, s.c.) at 12-h intervals for 3 days after surgery and meloxicam (0.2 mg/kg, s.c. – Metacam; Boehringer Ingelheim B.V., The Netherlands) at 24-h intervals for 2 days after surgery. Animals were monitored and weighed daily for 1 week after surgery and were allowed to recover for at least 8 days prior to operant training.

Alcohol self-administration under FR and PR schedules of reinforcement

The rats were trained and tested, as previously described (Lesscher *et al.*, 2015; Spoelder *et al.*, 2015a), in operant conditioning chambers (29.5 cm L, 24 cm W, 25 cm H; Med Associates, Georgia, VT, USA), situated in light- and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8-cm-wide retractable levers, placed 11.7 cm apart and 6 cm from the grid floor. A liquid dipper within a recessed magazine was situated between the levers. A cue light was present above each lever (28 V, 100 mA) and a house light (28 V, 100 mA) was located on the opposite wall. The position of the active and inactive levers was counterbalanced between rats. Pressing the active lever raised the dipper cup containing alcohol (0.1 mL, 20% v/v),

illuminated the cue light above the active lever and switched off the house light. Access to alcohol was terminated 10 s after head entry into the magazine, the cue light was extinguished and after a 5-s interval a new trial started. Pressing the inactive lever was recorded, but had no programmed consequences. The rats were tested for 3–4 days/week on every other day, and sessions lasted for 30 min. Alcohol consumption during self-administration sessions was calculated by weighing the container with alcohol underneath the liquid dipper before and after each session. To limit fluctuation of the alcohol concentration by evaporation, the alcohol solution was refreshed before each session. Experimental events and data recording were controlled using MED-PC for Windows.

The rats were habituated to the operant chamber for two 30-min sessions during which 15 alcohol rewards were freely available every other minute. After habituation, the rats were trained under a FR1 schedule of reinforcement for 11–15 sessions in which the rats obtained on average 27 ± 0.9 rewards/session. Microinfusions during FR1 sessions started after all rats acquired a response criterion of at least 10 rewards for seven consecutive sessions. After completion of the microinfusions for the FR1 schedule of reinforcement, the same rats were trained further and the response requirement was increased to a FR2, FR5 and FR10 schedule, during which each animal had to earn at least 10 rewards per FR schedule before progressing to the PR schedule of reinforcement. These requirements were set to ensure that the rats made at least 100 presses under the FR10 to obtain reliable response levels during PR sessions. The rats required on average 6 ± 0.3 sessions to obtain this criterion. Next, a linear PR schedule of reinforcement was introduced, in which two (PR2, i.e. 2, 4, 6, 8, 10) additional lever presses were required for each subsequent reward. This PR paradigm, rather than the commonly used exponential increase in the response requirement (Richardson & Roberts, 1996), was chosen based on the results of previous studies which showed that (i) alcohol non-preferring rats have low breakpoints, (ii) the required workload should be increased, however, before the sedative effects of alcohol begin to interfere with operant performance, (iii) alcohol is delivered in relatively small sizes (0.1 mL/reinforcement) with a slow absorption rate (Hodos, 1961; Ritz *et al.*, 1994; Brown *et al.*, 1998; Rodd *et al.*, 2003). Microinfusions during PR sessions started once responding stabilized, i.e. less than 25% variation in the number of reward deliveries over three consecutive sessions; this required on average 5 ± 0.5 sessions.

Microinfusions

Microinfusions were made, as previously described (Trezza *et al.*, 2011; Veeneman *et al.*, 2012), using 33 gauge injectors (Plastics One, Roanoke, VA, USA) that extended 2.0 mm below the guide cannulas and were connected to a 10-μl syringe. Using a microinfusion syringe pump (Harvard Apparatus, Holliston, MA, USA), bilateral microinfusions with alpha-flupenthixol (0.5 μL/side) were made over 60 s and the injectors were left in place for another 60 s to allow for drug diffusion. Immediately after the microinfusion procedure, the rats were placed into the operant chamber; the self-administration session started 5 min later. During 2 weeks prior to the start of the microinfusions of the FR1 sessions, the rats were habituated to the removal and replacement of the stylets in the cannulas every other day. In addition, each rat was habituated to the infusion procedure in which the rats received one sham control infusion (i.e. injectors were the same length as the guide cannulas, the pump motor was operated but the syringes were not driven) and one actual infusion with sterile physiological saline (0.9% NaCl). The effects of flupenthixol were examined using a within-subjects design in which each rat received all doses of flupenthixol according to a Latin square design. The rats were tested in the operant chambers every other day, and at least one re-baseline session without treatment was scheduled after each drug treatment to verify that response levels remained stable.

Histology

At the termination of the experiment, the rats were killed using an overdose of pentobarbital (200 mg/kg, IP) and ink was infused to aid visual localization of the infusion sites. The brains were removed, flash-frozen in methyl-butane isopentane (−80 °C) and subsequently stored at −80 °C. Coronal sections were sliced using a cryostat (40 μm), mounted, air-dried and stained with Cresyl violet. Microinjection sites were verified by light microscopy using a rat brain atlas (Paxinos & Watson, 2004). Data from rats with one or both cannulas placed outside of the target area were discarded from the analyses.

Drugs

Cis-(Z)-Flupenthixol-dihydrochloride (Sigma-Aldrich, Zwijndrecht, The Netherlands) was dissolved in sterile physiological saline (0.9% NaCl) to concentrations of 0, 3.75, 7.5 and 15 μg in 0.5 μL. Doses were based on prior reports (Vanderschuren *et al.*, 2005; Murray *et al.*, 2012; Veeneman *et al.*, 2012). Alcohol solutions (Klinipath, Duiven, The Netherlands) were freshly prepared once a week by diluting 99.5% alcohol with tap water to a final concentration of 20% (v/v).

Statistical analysis

Forty-six rats with correct cannula placements were used for statistical analyses for the FR1 sessions (NAcc shell: $n = 11$, NAcc core: $n = 16$, DLS: $n = 19$) (Fig. 1). Three animals with correct cannula placements (one in the NAcc shell and two in the NAcc core) did not fulfil the response criteria for the PR sessions and were, therefore, not tested under the PR schedule of reinforcement. The experiments were performed in two batches. Rats of the first batch were treated with three flupenthixol doses (0, 3.75, 7.5 μg/side) (FR1: NAcc shell: $n = 3$, NAcc core: $n = 9$, DLS: $n = 11$; PR: NAcc shell: $n = 3$, NAcc core: $n = 8$, DLS: $n = 11$) and rats of a second batch were treated with four flupenthixol doses (0, 3.75, 7.5, 15 μg/side) (FR1: NAcc shell: $n = 8$, NAcc core: $n = 7$, DLS: $n = 8$; PR: NAcc shell: $n = 7$, NAcc core: $n = 6$, DLS: $n = 8$). To combine both batches in the statistical analyses, we used linear mixed effects models (Verbeke & Molenberghs, 2000). The effect of flupenthixol on the number of lever presses and the rewards obtained during the FR and PR sessions were analysed in four bins of 7.5 min to assess the effects of flupenthixol over time. The session time (i.e. the period of active involvement in the session) was expressed as the duration from the start of the session to the time of the last active lever press. The response rate was calculated by dividing the number of active lever presses by the session time. All parameters were tested for normality with the Kolmogorov–Smirnov test prior to analyses. The latency to the first active lever press and the session time were log transformed prior to statistical analyses, and the inactive lever presses as well as the number of rewards and active lever presses over time bins were square root transformed to obtain a normal distribution of the data prior to statistical analyses. The number of lever presses and rewards, latencies and the response

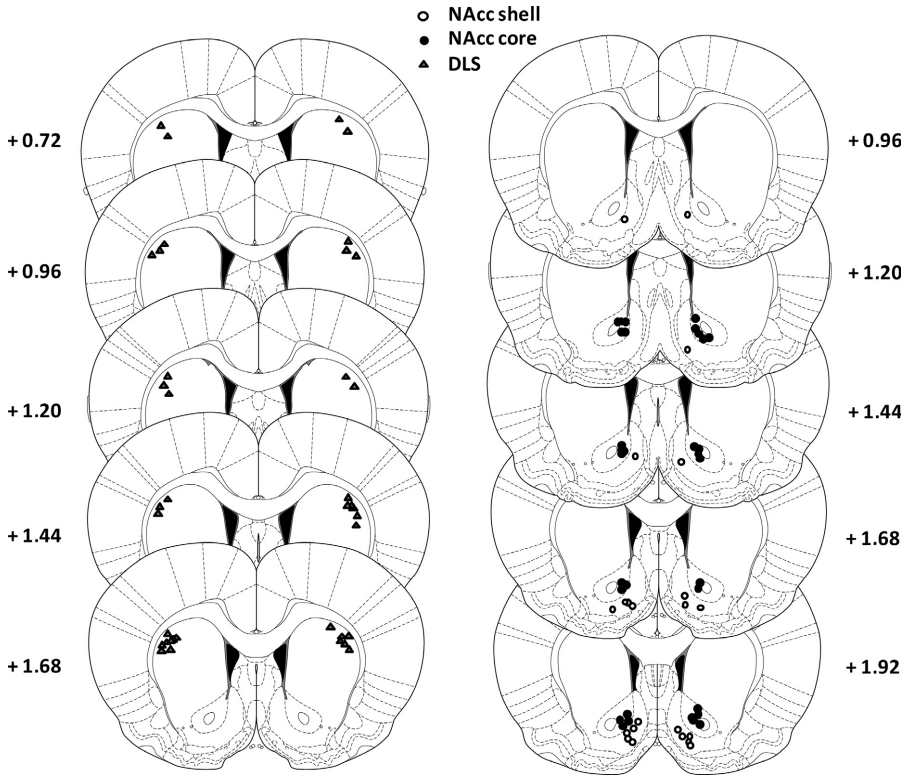

FIG. 1. Schematic representation of brain sections with microinjection placements in the NAcc shell (open circles), NAcc core (filled circles) and DLS (grey triangles). Numbers indicate the distances anterior to bregma in mm (adapted from Paxinos & Watson, 2004).

rates were analysed using linear mixed effects models in which dose, time bin and group (NAcc shell, NAcc core, DLS) were treated as independent variables. For all analyses, the covariance structure was explored and modelled appropriately. Alcohol intake and preference in the home cage was analysed using two-way repeated-measures ANOVAS with session or month as within-subject variables and group (NAcc shell, NAcc core, DLS) as between-subject variable. Mauchly's test of sphericity was used to test if variances of the differences between treatments were equal. If the assumption of sphericity had been violated, degrees of freedom were corrected using Huynh-Feldt estimates of sphericity to more conservative values; the corrected degrees of freedom are presented rounded to the nearest integer. The alcohol intake during operant sessions was calculated by an univariate ANOVA with treatment group as between-subjects factor. When significant main effects or interactions were detected, *post hoc* pairwise comparisons with a Bonferroni correction were made. All statistical analyses were conducted using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). The threshold for statistical significance was set at $P < 0.05$. Graphs were made using GRAPHPAD PRISM 6. All data are presented as mean ± SEM.

Results

Home-cage alcohol intake and preference

The alcohol intake and preference increased in the first month with 7 h alcohol access/day (intake: $F_{9,377\ \text{session}} = 8.84$, $P < 0.001$; preference: $F_{11,473\ \text{session}} = 15.14$, $P < 0.001$) (Fig. 2A and B). Increasing the alcohol exposure time in the second month to 24 h alcohol access/day further enhanced alcohol intake and preference (intake: $F_{2,43\ \text{month}} = 301.75$, $P < 0.001$; preference: $F_{2,43\ \text{month}} = 68.23$, $P < 0.001$). During the second month of voluntary alcohol consumption, alcohol intake remained stable ($F_{7,312\ \text{session}} = 1.70$, $P = 0.106$) (Fig. 2A), whereas the preference for alcohol continued to increase ($F_{8,350\ \text{session}} = 3.38$, $P = 0.001$) (Fig. 2B). The treatment groups (NAcc shell, NAcc core and DLS) did not differ in their alcohol intake and preference in the first month (intake: $F_{2,43\ \text{group}} = 1.16$, $P = 0.332$, $F_{18,377\ \text{session} \times \text{group}} = 1.12$, $P = 0.336$; preference: $F_{2,43\ \text{group}} = 0.99$, $P = 0.379$, $F_{22,473\ \text{session} \times \text{group}} = 1.21$, $P = 0.234$) or in the second month (intake: $F_{2,43\ \text{group}} = 1.00$, $P = 0.377$, $F_{15,312\ \text{session} \times \text{group}} = 1.39$, $P = 0.154$; preference: $F_{2,43\ \text{group}} = 1.33$, $P = 0.276$, $F_{16,350\ \text{session} \times \text{group}} = 1.39$, $P = 0.144$) (Fig. 2A and B).

Effects of alpha-flupenthixol infusions on responding for alcohol under a FR1 schedule of reinforcement

During FR1 sessions, the average level of alcohol intake of the rats under vehicle conditions was 0.62 ± 0.05 g/kg; this did not differ between the groups ($F_{2,43\ \text{group}} = 1.01$, $P = 0.373$). Alpha-flupenthixol affected responding for alcohol ($F_{3,116\ \text{dose}} = 10.84$, $P < 0.001$) in a group-dependent manner ($F_{2,47\ \text{group}} = 0.27$, $P = 0.764$; $F_{6,116\ \text{dose} \times \text{group}} = 2.70$, $P = 0.017$) (Fig. 3). The number of inactive responses was not affected by alpha-flupenthixol ($F_{3,119\ \text{dose}} = 2.53$, $P = 0.061$; $F_{2,49\ \text{group}} = 3.06$, $P = 0.056$; $F_{6,119\ \text{dose} \times \text{group}} = 1.19$, $P = 0.316$) (Fig. 3). Infusions with alpha-flupenthixol affected the number of rewards obtained ($F_{3,529\ \text{dose}} = 9.67$, $P < 0.001$) in a group-dependent fashion ($F_{2,53\ \text{group}} = 0.39$, $P = 0.679$; $F_{6,529\ \text{dose} \times \text{group}} = 3.28$, $P = 0.004$). The number of rewards obtained declined over the course of the session ($F_{3,265\ \text{time bin}} = 132.01$, $P < 0.001$; $F_{6,266\ \text{time bin} \times \text{group}} = 1.75$,

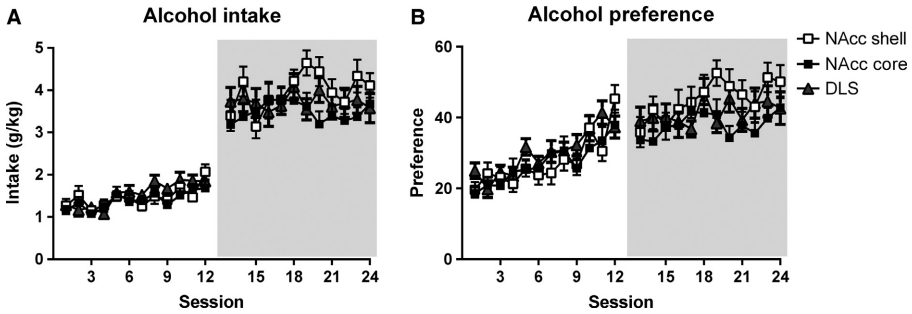

FIG. 2. Alcohol intake (A) and preference (B) during intermittent exposure to alcohol (20%, v/v) in the home cage preceding operant alcohol self-administration and microinfusions. (A) Alcohol intake increased over sessions in the first month (7 h access/day – white area), increased when access time was lengthened to 24 h/day and remained stable during sessions in the second month (24 h access/day – shaded area). (B) Alcohol preference increased over sessions in both months. Alcohol intake and preference did not differ between groups designated for NAcc shell, NAcc core and DLS infusions. Data are shown as mean + SEM per day per infusion group.

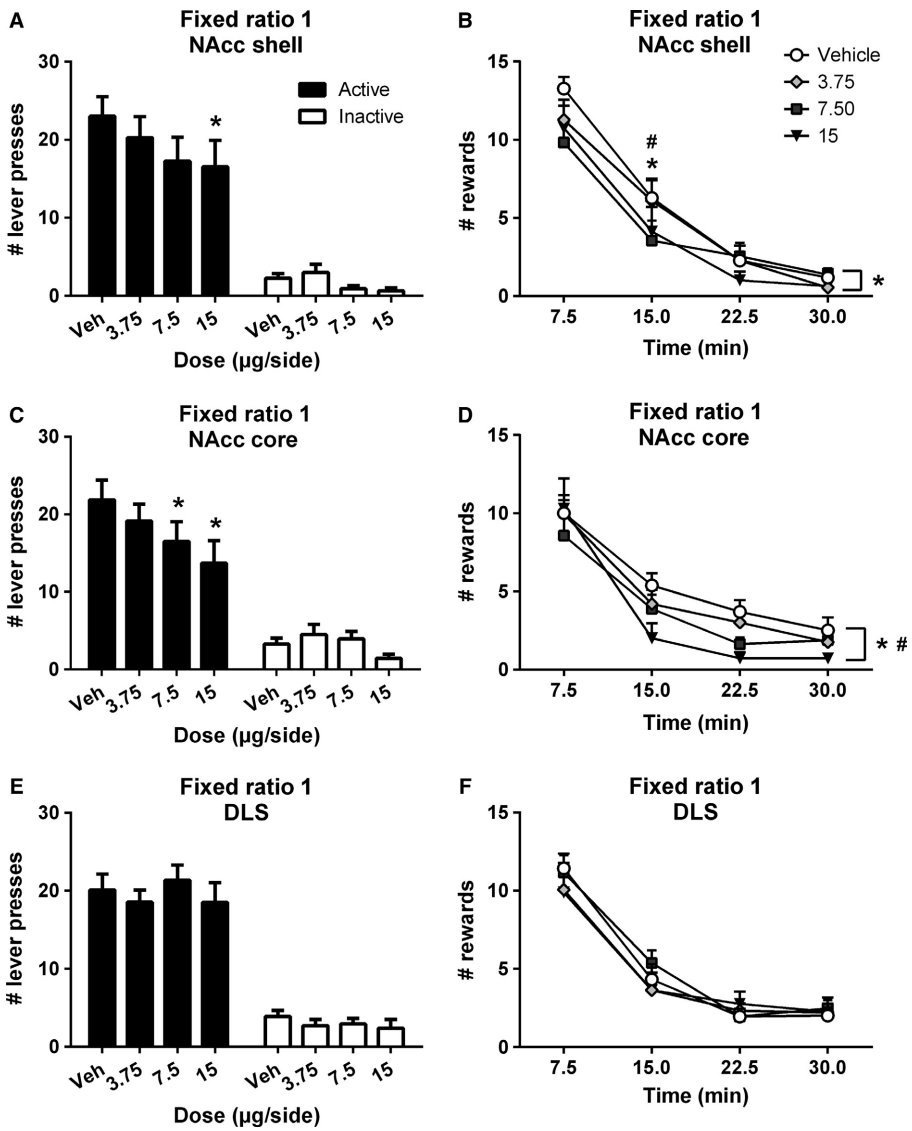

FIG. 3. Effects of alpha-flupenthixol infusions into the NAcc shell (A, B), NAcc core (C, D) and DLS (E, F) on responding for alcohol under a FR1 schedule of reinforcement. (A, C, E) Total number of active (black bars) and inactive (white bars) lever presses during alcohol self-administration. (B, D, F) Number of rewards obtained over time during alcohol self-administration. Alpha-flupenthixol infusions into the NAcc shell and core dose-dependently reduced the number of active lever presses as well as the number of rewards obtained over time during the session, whereas alpha-flupenthixol infusions into the DLS were ineffective. Data are presented as mean + SEM. Asterisk in (A, C and E) significantly different from vehicle (*post hoc* pairwise comparisons with Bonferroni correction). (B, D and F) *15 µg alpha-flupenthixol significantly different from vehicle; #7.5 µg alpha-flupenthixol significantly different from vehicle (*post hoc* pairwise comparisons with Bonferroni correction).

$P = 0.109$; $F_{9,540\ \text{time bin} \times \text{dose}} = 1.45$, $P = 0.164$). The effect of alpha-flupenthixol on the number of rewards obtained was dependent on both the session bin and the treatment group ($F_{18,540\ \text{dose} \times \text{group} \times \text{time bin}} = 1.66$, $P = 0.044$) (Fig. 3). The lever press latency was not influenced by alpha-flupenthixol ($F_{3,119\ \text{dose}} = 2.13$, $P = 0.100$; $F_{2,49\ \text{group}} = 0.29$, $P = 0.746$; $F_{6,119\ \text{dose} \times \text{group}} = 0.12$, $P = 0.994$) (Table 1). Alpha-flupenthixol affected session time in a group-dependent manner ($F_{3,67\ \text{dose}} = 2.73$, $P = 0.051$; $F_{2,86\ \text{group}} = 3.64$, $P = 0.030$; $F_{6,56\ \text{dose} \times \text{group}} = 1.56$, $P = 0.177$) (Table 2). The effect of infusions with alpha-flupenthixol on the response rate depended on the group ($F_{3,67\ \text{dose}} = 2.37$, $P = 0.078$; $F_{2,95\ \text{group}} = 2.53$, $P = 0.085$; $F_{6,56\ \text{dose} \times \text{group}} = 2.81$, $P = 0.018$). We subsequently analysed the data separately for each group (i.e., brain structure).

NAcc shell

Infusion of alpha-flupenthixol into the NAcc shell dose dependently decreased alcohol self-administration ($F_{3,30\ \text{dose}} = 4.22$, $P = 0.013$). *Post hoc* analyses indicated that responding for alcohol was significantly reduced after treatment with 15 μg alpha-flupenthixol ($P = 0.018$) (Fig. 3A). Inactive lever presses were not affected by alpha-flupenthixol infusions ($F_{3,30\ \text{dose}} = 2.80$, $P = 0.056$) (Fig. 3A). The number of rewards obtained declined over the course of the session ($F_{3,25\ \text{time bin}} = 146.60$, $P < 0.001$), and it was significantly reduced by alpha-flupenthixol ($F_{3,43\ \text{dose}} = 5.71$, $P = 0.002$). *Post hoc* analyses showed that the number of rewards was reduced after infusion of 15 μg alpha-flupenthixol ($P = 0.003$) (Fig. 3B). The effect of alpha-flupenthixol on rewards obtained was dependent on the time bin ($F_{9,79\ \text{time bin} \times \text{dose}} = 2.12$, $P = 0.037$); *post hoc* analyses indicated that less rewards were obtained after infusion of 7.5 μg ($P = 0.008$) and 15 μg ($P = 0.024$) alpha-flupenthixol in the second time bin (Fig. 3B). The onset of responding was unaffected by alpha-flupenthixol treatment (Table 1). While alpha-flupenthixol affected session time, no significant *post hoc* differences were apparent after alpha-flupenthixol treatment (Table 2). Alpha-flupenthixol affected the response rate; *post hoc* analyses indicated a significant reduction after infusion of 7.5 μg alpha-flupenthixol (Table 3).

NAcc core

Infusion of alpha-flupenthixol into the NAcc core dose dependently decreased alcohol self-administration ($F_{3,14\ \text{dose}} = 34.58$, $P < 0.001$); *post hoc* analyses revealed significant reductions in the

TABLE 1. Latency to the first active lever press in the session (sec)

		Dose effect	Vehicle	3.75	7.5	15
FR1	Shell	$F_{3,41\ \text{dose}} = 0.194$, $P = 0.900$	9.4 ± 4.4	20.2 ± 9.0	16.5 ± 8.3	9.6 ± 3.5
	Core	$F_{3,40\ \text{dose}} = 1.390$, $P = 0.260$	8.4 ± 3.0	15.2 ± 7.6	22.7 ± 7.9	23.1 ± 16.2
	DLS	$F_{3,48\ \text{dose}} = 1.289$, $P = 0.289$	12.5 ± 3.9	23.1 ± 12.1	48.8 ± 19.6	16.7 ± 6.6
PR	Shell	$F_{3,24\ \text{dose}} = 0.778$, $P = 0.518$	6.4 ± 3.1	8.9 ± 2.7	8.8 ± 5.4	9.8 ± 5.8
	Core	$F_{3,18\ \text{dose}} = 2.973$, $P = 0.060$	3.1 ± 0.5	6.9 ± 3.0	3.8 ± 0.8	2.0 ± 0.1
	DLS	$F_{3,30\ \text{dose}} = 1.616$, $P = 0.206$	24.0 ± 13.9	22.8 ± 9.9	8.3 ± 2.4	16.4 ± 6.7

Data are presented as mean ± SEM.

TABLE 2. Session time (min)

		Dose effect	Vehicle	3.75	7.5	15
FR1	Shell	$F_{3,12\ \text{dose}} = 3.497$, $P = 0.050$	21.6 ± 1.7	20.6 ± 2.3	22.0 ± 2.4	15.5 ± 3.2
	Core	$F_{3,14\ \text{dose}} = 3.038$, $P = 0.065$	26.0 ± 1.0	24.0 ± 1.6	25.8 ± 0.7	19.9 ± 4.1
	DLS	$F_{3,30\ \text{dose}} = 0.781$, $P = 0.514$	24.0 ± 1.8	25.3 ± 1.0	25.9 ± 1.1	24.7 ± 1.8
PR	Shell	$F_{3,10\ \text{dose}} = 6.651$, $P = 0.010$	26.4 ± 0.7	16.0 ± 2.8*	16.0 ± 3.8†	10.9 ± 3.4†
	Core	$F_{3,12\ \text{dose}} = 17.134$, $P < 0.001$	25.4 ± 1.3	22.0 ± 1.4†	19.2 ± 2.0	14.8 ± 3.2*
	DLS	$F_{3,18\ \text{dose}} = 4.976$, $P = 0.011$	22.6 ± 1.6	23.4 ± 1.7	21.5 ± 2.1	14.4 ± 3.6*

Data are presented as mean ± SEM. *Significantly different from vehicle (*post hoc* analysis with Bonferroni correction; $P = 0.045$). †Trend towards significant difference from vehicle (*post hoc* analysis with Bonferroni correction; $0.070 < P < 0.085$).

TABLE 3. Response rate

		Dose effect	Vehicle	3.75	7.5	15
FR1	Shell	$F_{3,19\ \text{dose}} = 7.982$, $P = 0.001$	1.1 ± 0.1	1.1 ± 0.1	0.8 ± 0.1*	1.2 ± 0.2
	Core	$F_{3,18\ \text{dose}} = 1.171$, $P = 0.349$	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.3
	DLS	$F_{3,45\ \text{dose}} = 1.875$, $P = 0.147$	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
PR	Shell	$F_{3,14\ \text{dose}} = 1.016$, $P = 0.414$	4.3 ± 0.5	6.0 ± 1.3	6.1 ± 1.7	6.6 ± 1.3
	Core	$F_{3,19\ \text{dose}} = 1.478$, $P = 0.253$	5.4 ± 0.5	5.3 ± 0.7	4.3 ± 0.6	5.5 ± 1.2
	DLS	$F_{3,11\ \text{dose}} = 2.965$, $P = 0.079$	5.4 ± 0.6	3.7 ± 0.5	5.0 ± 1.2	3.1 ± 0.8

Data are presented as mean ± SEM. *Significantly different from vehicle (*post hoc* analysis with Bonferroni correction; $P = 0.050$).

number of active responses after infusion of 7.5 μg ($P = 0.033$) and 15 μg alpha-flupenthixol ($P < 0.001$) (Fig. 3C). Inactive lever presses were not affected by alpha-flupenthixol treatment ($F_{3,55\ dose} = 1.02$, $P = 0.392$) (Fig. 3C). The number of obtained rewards declined over the course of the session ($F_{3,83\ time\ bin} = 35.85$, $P < 0.001$) and alpha-flupenthixol decreased the number of rewards ($F_{3,173\ dose} = 10.44$, $P < 0.001$), independent of time in the session ($F_{9,178\ time\ bin \times dose} = 1.45$, $P = 0.171$). *Post hoc* analyses showed that the number of rewards was reduced after infusion of 7.5 μg ($P < 0.001$) and 15 μg alpha-flupenthixol ($P < 0.001$) (Fig. 3D). The onset of responding and the response rate were unaffected by alpha-flupenthixol infusions (Tables 1 and 3). Alpha-flupenthixol treatment resulted in a trend towards a reduced session time (Table 2).

Dorsolateral striatum

Infusion of alpha-flupenthixol into the DLS had no effect on the number of active ($F_{3,47\ dose} = 1.87$, $P = 0.147$) and inactive lever presses ($F_{3,29\ dose} = 1.55$, $P = 0.223$) (Fig. 3E). The number of obtained rewards declined over the course of the session ($F_{3,40\ time\ bin} = 37.69$, $P < 0.001$), independent of the alpha-flupenthixol dose ($F_{3,52\ dose} = 1.30$, $P = 0.283$; $F_{9,84\ time\ bin \times dose} = 1.39$, $P = 0.207$) (Fig. 3F). The onset of responding, session time and the response rate were unaffected by alpha-flupenthixol treatment in the DLS (Tables 1–3).

Effects of alpha-flupenthixol infusions on responding for alcohol under a PR schedule of reinforcement

During PR sessions, the average level of alcohol intake of the rats under vehicle conditions was 0.31 ± 0.02 g/kg; this did not differ between the treatment groups ($F_{2,40\ group} = 1.67$, $P = 0.202$). Infusion of alpha-flupenthixol affected responding for alcohol under the PR schedule of reinforcement ($F_{3,80\ dose} = 14.68$, $P < 0.001$; $F_{2,44\ group} = 2.05$, $P = 0.141$; $F_{6,81\ dose \times group} = 1.22$, $P = 0.305$) (Fig. 4). The number of inactive responses was also altered by alpha-flupenthixol infusion ($F_{3,110\ dose} = 3.03$, $P = 0.033$), and this was dependent on the group ($F_{2,45\ group} = 9.76$, $P < 0.001$; $F_{6,110\ dose \times group} = 3.27$, $P = 0.005$) (Fig. 4). When responding over time in the session was analysed, we found that alpha-flupenthixol affected the number of active lever presses ($F_{3,453\ dose} = 21.30$, $P < 0.001$; $F_{2,45\ group} = 2.22$, $P = 0.120$; $F_{6,453\ dose \times group} = 1.55$, $P = 0.160$; $F_{18,476\ dose \times group \times time\ bin} = 0.81$, $P = 0.694$). The number of active lever presses declined over the course of the session ($F_{3,188\ time\ bin} = 102.05$, $P < 0.001$; $F_{6,190\ time\ bin \times group} = 1.45$, $P = 0.197$; $F_{9,477\ time\ bin \times dose} = 1.65$, $P = 0.098$) (Fig. 4). Infusion of alpha-flupenthixol affected the number of obtained rewards ($F_{3,417\ dose} = 21.15$, $P < 0.001$; $F_{2,51\ group} = 2.68$, $P = 0.078$; $F_{6,417\ dose \times group} = 1.60$, $P = 0.145$; $F_{18,437\ dose \times group \times time\ bin} = 1.24$, $P = 0.222$). The number of obtained rewards declined over the course of the session ($F_{3,185\ time\ bin} = 285.28$, $P < 0.001$), which was different between groups ($F_{6,187\ time\ bin \times group} = 2.56$, $P = 0.021$) and tended to differ between doses ($F_{9,437\ time\ bin \times dose} = 1.86$, $P = 0.056$) (Fig. 4). The lever press latency was not influenced by alpha-flupenthixol ($F_{3,109\ dose} = 1.59$, $P = 0.195$; $F_{2,49\ group} = 2.78$, $P = 0.072$; $F_{6,109\ dose \times group} = 0.45$, $P = 0.843$) (Table 1). Infusion of alpha-flupenthixol decreased the session time ($F_{3,23\ dose} = 10.55$, $P < 0.001$; $F_{2,42\ group} = 1.72$, $P = 0.192$; $F_{6,26\ dose \times group} = 1.54$, $P = 0.204$) (Table 2). The response rate was not affected by alpha-flupenthixol ($F_{3,55\ dose} = 0.14$, $P = 0.934$; $F_{2,45\ group} = 1.43$, $P = 0.250$; $F_{6,56\ dose \times group} = 2.00$, $P = 0.082$) (Table 3). We subsequently analysed the data separately for each group (i.e., brain structure).

NAcc shell

Infusion of alpha-flupenthixol into the NAcc shell dose dependently decreased responding for alcohol ($F_{3,27\ dose} = 10.40$, $P < 0.001$). *Post hoc* analyses indicated that infusion of 3.75 μg ($P = 0.001$), 7.5 μg ($P < 0.001$) and 15 μg alpha-flupenthixol ($P = 0.001$) reduced the number of active lever presses (Fig. 4A). Inactive lever presses were not altered by alpha-flupenthixol ($F_{3,26\ dose} = 1.08$, $P = 0.374$) (Fig. 4A). Analyses of the number of active lever presses in time showed that responding declined during the session ($F_{3,60\ time\ bin} = 91.57$, $P < 0.001$). Alpha-flupenthixol treatment decreased the number of active lever presses throughout the session ($F_{3,59\ dose} = 8.46$, $P < 0.001$; $F_{9,41\ time\ bin \times dose} = 1.18$, $P = 0.336$); *post hoc* analyses indicated that the number of active lever presses was reduced after all alpha-flupenthixol doses infused: 3.75 μg ($P = 0.014$), 7.5 μg ($P < 0.001$) and 15 μg ($P < 0.001$) (Fig. 4B). Analyses of the number of obtained rewards in time resembled the results of the active lever presses, indicating a decrease in the number of rewards over the session ($F_{3,66\ time\ bin} = 133.58$, $P < 0.001$), whereby alpha-flupenthixol caused an overall reduction in the number of rewards ($F_{3,105\ dose} = 12.05$, $P < 0.001$; $F_{9,108\ time\ bin \times dose} = 0.79$, $P = 0.630$). *Post hoc* analyses showed that the number of obtained rewards was reduced after infusion of alpha-flupenthixol at all doses tested: 3.75 μg ($P = 0.001$), 7.5 μg ($P < 0.001$) and 15 μg ($P < 0.001$) (Fig. 4C). The onset of responding was unaffected by alpha-flupenthixol treatment (Table 1). However, infusion of alpha-flupenthixol reduced the session time. *Post hoc* analyses indicated a shorter session time after treatment with 3.75 μg alpha-flupenthixol, and a trend towards a shorter session time for the 7.5 μg and 15 μg doses (Table 2). The response rate was not altered by alpha-flupenthixol (Table 3).

NAcc core

Infusion of alpha-flupenthixol into the NAcc core decreased responding for alcohol ($F_{3,7\ dose} = 66.66$, $P < 0.001$) at doses of 7.5 μg ($P = 0.048$) and 15 μg ($P = 0.001$) (Fig. 4D). Inactive lever presses were unaffected by alpha-flupenthixol infusion ($F_{3,9\ dose} = 2.84$, $P = 0.095$) (Fig. 4D). The number of active lever presses declined over the session ($F_{3,78\ time\ bin} = 25.96$, $P < 0.001$) and alpha-flupenthixol treatment decreased the number of active lever presses throughout the session ($F_{3,143\ dose} = 7.73$, $P < 0.001$; $F_{9,149\ time\ bin \times dose} = 0.52$, $P = 0.857$). *Post hoc* analyses indicated that the number of active lever presses was reduced after 7.5 μg ($P = 0.001$) and 15 μg alpha-flupenthixol ($P = 0.001$) (Fig. 4E). Likewise, the number of rewards obtained decreased over the session ($F_{3,85\ time\ bin} = 88.17$, $P < 0.001$). Alpha-flupenthixol treatment decreased the number of rewards throughout the session ($F_{3,141\ dose} = 6.49$, $P < 0.001$; $F_{9,145\ time\ bin \times dose} = 0.95$, $P = 0.482$). *Post hoc* analyses showed that the number of obtained rewards was reduced after infusion of 7.5 μg ($P = 0.005$) and 15 μg alpha-flupenthixol ($P = 0.001$) (Fig. 4F). The onset of responding was not changed by alpha-flupenthixol treatment (Table 1). Infusion of alpha-flupenthixol reduced the session time. *Post hoc* analyses indicated a shorter session time after the infusion of 15 μg alpha-flupenthixol, and a trend towards significance for the 3.75 μg dose (Table 2). The response rate was unaffected by alpha-flupenthixol (Table 3).

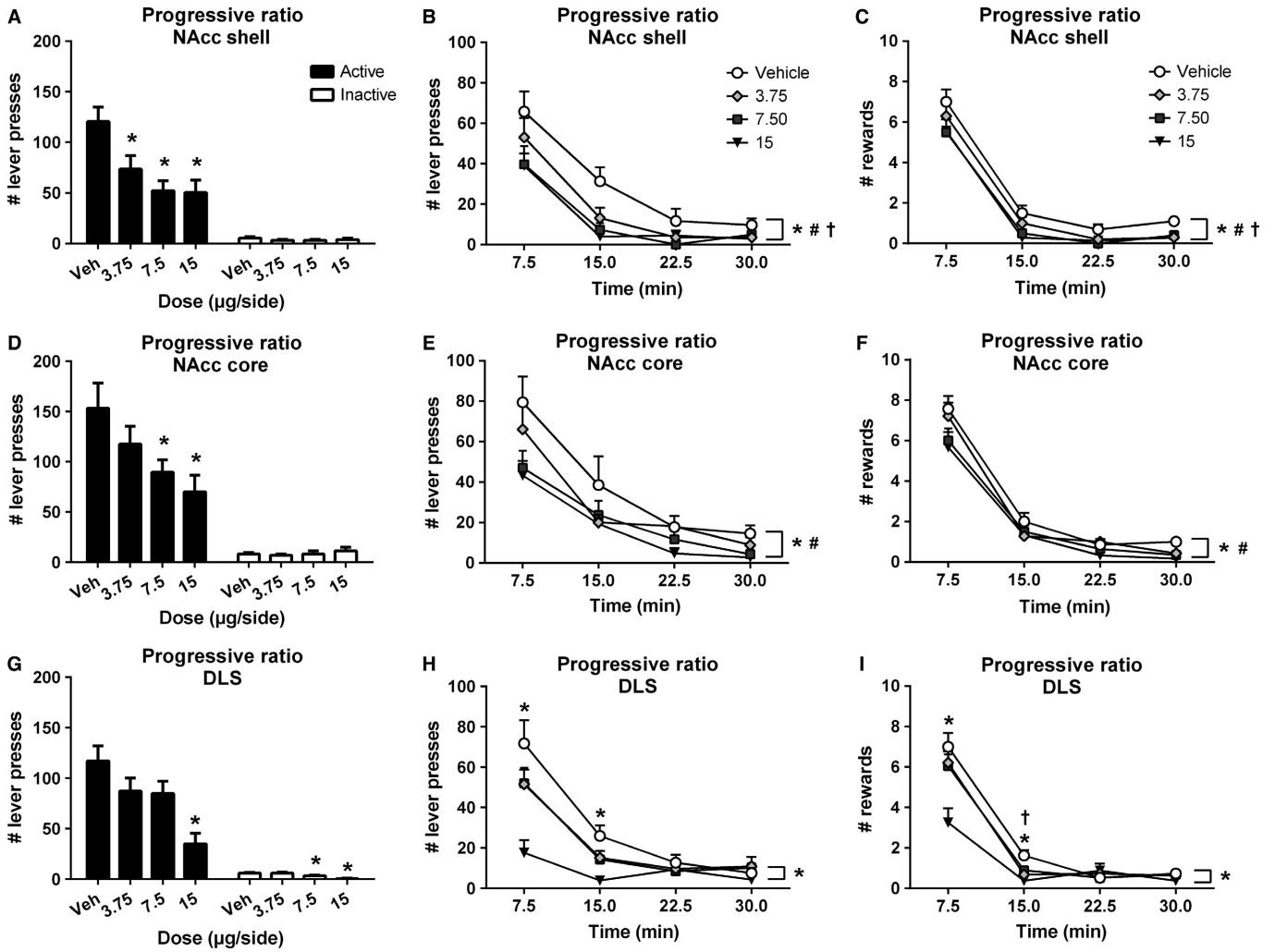

FIG. 4. Effects of alpha-flupenthixol infusions into the NAcc shell (A–C), NAcc core (D–F) and DLS (G–I) on responding for alcohol under a PR schedule of reinforcement. (A, D, G) Total number of active (black bars) and inactive (white bars) lever presses during alcohol self-administration. (B, E, H) Total number of active lever presses over time during alcohol self-administration. (C, F, I) Number of rewards obtained over time during alcohol self-administration. Alpha-flupenthixol infusions into the NAcc shell, NAcc core and DLS dose-dependently reduced responding for alcohol and decreased the number of rewards obtained. Alpha-flupenthixol had no effect on the number of inactive lever presses when infused into the NAcc shell and NAcc core, but reduced the number of inactive lever presses when infused into the DLS. Data are presented as mean + SEM. Asterisk in (A, D and G) significantly different from vehicle (*post hoc* pairwise comparisons with Bonferroni correction). (B, C, E, F, H and I) *15 µg alpha-flupenthixol significantly different from vehicle; #7.5 µg alpha-flupenthixol significantly different from vehicle; †3.75 µg alpha-flupenthixol significantly different from vehicle (*post hoc* pairwise comparisons with Bonferroni correction).

Dorsolateral striatum

Infusion of alpha-flupenthixol into the DLS reduced the number of active ($F_{3,12\ \text{dose}} = 6.92$, $P = 0.005$) and inactive lever presses ($F_{3,47\ \text{dose}} = 9.51$, $P < 0.001$). *Post hoc* analyses showed that the number of active lever presses was decreased after infusion of 15 µg alpha-flupenthixol ($P = 0.018$) and the number of inactive lever presses was reduced after infusion of 7.5 µg ($P = 0.020$) and 15 µg alpha-flupenthixol ($P < 0.001$) (Fig. 4G). Responding declined over the course of the session ($F_{3,241\ \text{time bin}} = 53.24$, $P < 0.001$), and it was decreased by alpha-flupenthixol treatment ($F_{3,245\ \text{dose}} = 4.96$, $P = 0.002$) at the dose of 15 µg ($P = 0.001$) (Fig. 4H). Moreover, the effect of alpha-flupenthixol was dependent on time in the session ($F_{9,241\ \text{time bin} \times \text{dose}} = 1.92$, $P = 0.050$); *post hoc* analyses showed a significant reduction in active lever presses after 15 µg alpha-flupenthixol treatment during the first ($P < 0.001$) and the second time bin ($P = 0.011$) (Fig. 4H). The number of rewards obtained decreased during the session ($F_{3,84\ \text{time bin}} = 118.88$, $P < 0.001$); this was affected by alpha-flupenthixol infusions ($F_{3,200\ \text{dose}} = 4.64$, $P = 0.004$). *Post hoc* analyses indicated that alpha-flupenthixol decreased the number of rewards at a dose of 15 µg ($P = 0.003$) (Fig. 4I). The reduction in the number of rewards evoked by alpha-flupenthixol infusions into the DLS was dependent on the time in the session ($F_{9,209\ \text{time bin} \times \text{dose}} = 2.97$, $P = 0.002$). *Post hoc* analyses revealed that the number of rewards was reduced after treatment with 15 µg alpha-flupenthixol during the first ($P = 0.001$) and second time bin ($P = 0.004$). In addition, in the second time bin, the dose of 3.75 µg alpha-flupenthixol reduced the number of rewards as well ($P = 0.001$), and there was a trend towards a reduction for the 7.5 µg dose ($P = 0.053$) (Fig. 4I). The onset of responding was not altered by alpha-flupenthixol treatment (Table 1). The rats ceased responding earlier in the session after infusion of 15 µg alpha-flupenthixol (Table 2). The response rate was not affected by alpha-flupenthixol (Table 3).

Discussion

In this study, we found that dopamine receptor blockade in the NAcc shell and core reduced responding for alcohol under both FR1 and PR schedules of reinforcement. Alpha-flupenthixol treatment in the NAcc core reduced responding under both schedules at similar doses. In the NAcc shell, however, responding under the PR schedule of reinforcement was reduced at lower doses of alpha-flupenthixol than responding under the FR1 schedule. Infusion of alpha-flupenthixol into the DLS decreased responding for alcohol under the PR, but not the FR1 schedule of reinforcement. The alpha-flupenthixol-induced reductions in responding were associated with an earlier termination of responding for alcohol. Together, these findings indicate that alcohol reinforcement relies upon coordinated dopamine activity throughout the striatum, whereby different subregions play distinct roles in alcohol-directed behaviour.

The role of NAcc shell dopamine in responding for alcohol

Previous studies assessing the effects of treatment with dopamine receptor antagonists into the ventral striatum have reported reductions in alcohol self-administration (Hodge *et al.*, 1992, 1997; Rassnick *et al.*, 1992; Samson *et al.*, 1993; Czachowski *et al.*, 2001; Samson & Chappell, 2004). However, these studies investigated responding under a FR schedule of reinforcement only and did not distinguish between the NAcc shell and core. In this study, alpha-flupenthixol infusion into the NAcc shell decreased responding for alcohol, which is in agreement with the previously reported role of the NAcc shell in the reinforcing properties of food and drug rewards (Ikemoto *et al.*, 1997; Pecina & Berridge, 2000; Di Chiara, 2002; Rodd-Henricks *et al.*, 2002; Bassareo *et al.*, 2003; Ikemoto, 2003). Alcohol is reliably self-administered into the NAcc shell, but not the NAcc core (Engleman *et al.*, 2009). Moreover, in a recent study, infusion of dopamine receptor antagonists into the NAcc shell, but not the NAcc core, reduced responding for self-infusions of alcohol in the posterior ventral tegmental area (Ding *et al.*, 2015). Importantly, alpha-flupenthixol infused into the NAcc shell decreased responding for alcohol under the PR schedule at a four-fold lower dose as compared to the FR1 schedule of reinforcement. Since the response requirement under an FR1 schedule is minimal, responding under this schedule is thought to reflect consummatory aspects of self-administration, whereas PR schedules, because of their increasing response requirement, address processes related to the incentive motivational properties of rewards (Katz, 1990; Markou *et al.*, 1993; Richardson & Roberts, 1996; Arnold & Roberts, 1997). Hence, the current findings suggest that dopamine in the NAcc shell modulates the motivational aspects of responding for alcohol. This finding resonates well with our previous report on cocaine self-administration (Veeneman *et al.*, 2012). Thus, NAcc shell dopamine may mediate the motivation to obtain substances of abuse from different pharmacological classes.

The role of NAcc core dopamine in responding for alcohol

Infusion of alpha-flupenthixol into the NAcc core reduced responding for alcohol at comparable doses under both FR1 and PR schedules of reinforcement. These observations suggests that NAcc core dopamine plays a general role in alcohol reinforcement, which may be related to either the positive subjective properties of the substance or the role of alcohol-associated cues in self-administration. With regard to the latter possibility, the NAcc core has been widely implicated in the control of conditioned cues over behaviour, such that the value of reward-related stimuli is integrated to influence the organization of motor activity (Parkinson *et al.*, 1999; Ito *et al.*, 2004; Day *et al.*, 2007; Ambroggi *et al.*, 2011; Flagel *et al.*, 2011; Spoelder *et al.*, 2015b; West & Carelli, 2016; for reviews see Cardinal *et al.*, 2002; Floresco, 2015). During alcohol self-administration, the animals are exposed to different alcohol-associated cues (e.g. transportation to the operant chambers, the context of the chambers, presentation of the levers, and the smell of alcohol) as well as response contingent cues (rising of the dipper cup, illumination of the cue light). These cues by themselves can induce dopamine release within the NAcc (Weiss *et al.*, 1993; Katner *et al.*, 1996; Gonzales & Weiss, 1998; Melendez *et al.*, 2002). Importantly, previous studies have shown that the rise in dopamine levels in the ventral striatum is associated with the presentation of alcohol-associated cues and the anticipation of alcohol reinforcement, rather than with the concentration of alcohol in the ventral striatum (Weiss *et al.*, 1993; Melendez *et al.*, 2002; Doyon *et al.*, 2003, 2005). Interestingly, infusion of dopamine D2 receptor antagonists into the NAcc core has been shown to reduce responding for alcohol, but not its actual consumption (Czachowski *et al.*, 2001; Samson & Chappell, 2004). It is, therefore, likely that blockade of dopaminergic neurotransmission in the NAcc core interfered with the processing of alcohol-related cues to modulate alcohol self-administration, as the conditioned cues are largely similar during FR and PR sessions.

The role of DLS dopamine in responding for alcohol

For the DLS, we observed a reduction in responding for alcohol after alpha-flupenthixol infusions under the PR schedule, but not the FR1 schedule of reinforcement. These findings suggest that dopamine in the DLS is involved in the motivational aspects of responding for alcohol. However, unlike dopamine in the NAcc shell, this only becomes apparent when high levels of effort are required to obtain alcohol. With regard to its involvement in addictive behaviour, the DLS has been implicated in drug-seeking and drug-taking habits, which is thought to facilitate to the development of compulsive drug seeking after extended substance abuse (Yin *et al.*, 2004; Everitt & Robbins, 2005; Corbit *et al.*, 2012; Barker & Taylor, 2014). Indeed, dopaminergic neurotransmission in the DLS has been shown to contribute to habitual responding for alcohol (Corbit *et al.*, 2014), but the role of DLS dopamine in alcohol-reinforced behaviour has not been studied previously. In the present experiments, the effect of alpha-flupenthixol was first assessed under the FR1 schedule and subsequently under the PR schedule of reinforcement in the same animals. One may, therefore, reason that the effects of alpha-flupenthixol under the PR schedule are the result of the development of habitual patterns of responding with prolonged operant training and alcohol consumption. However, we think that this explanation is not likely to account for the present findings, because relatively few operant sessions separated the infusions during FR1 and PR sessions in comparison to the alcohol sessions in the home cage and operant chamber prior to infusions during the FR1 schedule. Importantly, the current findings are, in part, in line with the effects obtained during cocaine self-administration in which responding increased during the FR1 schedule but decreased during the PR schedule of reinforcement upon systemic and intra-DLS dopamine receptor blockade (Ettenberg *et al.*, 1982; Caine & Koob, 1994; Bourland & French, 1995; Vanderschuren *et al.*, 2005; Veeneman *et al.*, 2012). Taken together, these findings suggest

involvement of DLS dopamine in the motivational aspects of drug self-administration.

Behavioural analysis of the role of striatal dopamine in alcohol reinforcement

To gain more insight into the mechanisms by which striatal dopamine modulates responding for alcohol, we determined the effects of alpha-flupenthixol on the response onset, the duration of responding (i.e. session time) and the response rate. Treatment with alpha-flupenthixol leads to an earlier termination of responding in the session. These effects were especially pronounced for the PR schedule of reinforcement, which emphasizes the involvement of striatal dopamine in the motivation to obtain alcohol, especially when a large effort is required. These findings concur with previous reports on the duration of responding for alcohol after systemic (Pfeffer & Samson, 1988; Aberman *et al.*, 1998; Czachowski *et al.*, 2002) and local infusions of dopamine receptor antagonists (Samson *et al.*, 1993; Hodge *et al.*, 1997; Czachowski *et al.*, 2001). Intra-striatal administration of dopamine receptor antagonists (Fowler, 1990; Salamone, 1992; Baldo *et al.*, 2002) has previously been reported to reduce motor activity, which could also explain the earlier response termination. However, in this study, dopamine receptor blockade did not affect the onset of responding, had no major effects on the response rate and did not affect inactive lever presses, except after alpha-flupenthixol infusions into the DLS under the PR schedule. Moreover, the alpha-flupenthixol doses used were in the same range as the doses that increased responding for cocaine upon intra-DLS infusion under a FR schedule of reinforcement and did not alter responding for food (Vanderschuren *et al.*, 2005; Veeneman *et al.*, 2012; Willuhn *et al.*, 2012). Taken together, it is, therefore, not likely that alpha-flupenthixol caused a general decrease in motor function in this study. The reduction in inactive lever presses upon alpha-flupenthixol infusions into the DLS under the PR schedule may then be secondary to the reduction in active responses, as DLS dopamine appears to be particularly involved in alcohol reinforcement when the response requirement is high.

Methodological considerations

For this study, we selected the middle range of alcohol consuming rats, with relatively low inter-individual variation in alcohol ingestion (Spoelder *et al.*, 2015a). This is a limitation of the study, because conclusions regarding the role of striatal dopamine in alcohol reinforcement may not apply to high or low alcohol drinking individuals. At the same time, this middle group can be thought of as a representation of moderate drinkers, which probably constitutes the majority of alcohol drinking humans, as opposed to the abstainers and excessive drinkers. Important in this respect is the question whether these medium alcohol drinking rats can be considered 'addicted'. We previously reported that the 50% medium drinking rats do not display escalation of alcohol intake (a key characteristic of addiction-like behaviour), upon extension of alcohol access from 7 h to 24 h in the second month of IAA home-cage drinking, whereas the upper 25% high alcohol drinking rats do (Spoelder *et al.*, 2015a). However, more recent data from our laboratory show that, with prolonged alcohol access, the medium drinking rats do show reduced sensitivity to conditioned suppression (cf. Vanderschuren and Everitt, 2004) of alcohol seeking (M. Spoelder, S. Pol, B.S.G. Janssen, A.M. Baars, L.J.M.J. Vanderschuren & H.M.B. Lesscher, submitted.). These findings suggest that medium drinkers are a relevant group of individuals that, with extended exposure to alcohol, do develop signs of AUD-like behaviour.

Conclusions

In sum, this study provides novel insight into the differential role of dopamine within subregions of the striatal complex in alcohol-directed behaviour. Dopaminergic neurotransmission in the NAcc shell contributes to the motivational properties of alcohol, while NAcc core dopamine modulates alcohol reinforcement in general, perhaps by influencing the role of alcohol-associated cues in alcohol self-administration. DLS dopamine also influences the motivation to obtain alcohol, but only when this requires high levels of effort. Together, these findings show that alcohol reinforcement relies on coordinated dopaminergic activity within the striatum.

Conflict of interest

We declare no competing financial interests.

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We dedicate this article to the memory of our department chair Prof. Frauke Ohl, who passed away on 28 January 2016. We remember her as an inspiring colleague, mentor and friend. We thank Kathy C.G. de Git for practical assistance.

Abbreviations

AUD, alcohol use disorder; DLS, dorsolateral striatum; IAA, intermittent alcohol access; NAcc, nucleus accumbens; VTA, ventral tegmental area.

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