

# Nucleus accumbens core and pathogenesis of compulsive checking

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To investigate the role of the nucleus accumbens core (NAc) in the development of quinpirole-induced compulsive checking, rats received an excitotoxic lesion of NAc or sham lesion and were injected with quinpirole (0.5 mg/kg) or saline; development of checking behavior was monitored for 10 biweekly tests. The results showed that even after the NAc lesion, quinpirole still induced compulsive checking, suggesting that the pathogenic effects produced by quinpirole lie outside the NAc. Although the NAc lesion did not prevent the induction of compulsive checking, it altered how quickly it develops, suggesting that the NAc normally contributes toward the induction of compulsive checking. Saline-treated rats with an NAc lesion were hyperactive, but did not develop compulsive checking, indicating that hyperactivity by itself is not sufficient for the pathogenesis of compulsive checking. It is proposed that compulsive checking is the exaggerated output of a security motivation system and that the NAc serves as a neural hub for coordinating the orderly activity of neural modules of this motivational system. Evidence is considered suggesting

that the neurobiological condition for the pathogenesis of compulsive checking is two-fold: activation of dopamine D2/D3 receptors without concurrent stimulation of D1-like receptors and long-term plastic changes related to quinpirole-induced sensitization. *Behavioural Pharmacology* 26:200–216 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

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## Introduction

Considerable evidence supports the theory that the pathophysiology of obsessive-compulsive disorder (OCD) involves overactive functional loops comprising cortico-striato-pallido-thalamo-cortical connections (Modell *et al.*, 1989; Wise and Rapoport, 1989; Saxena *et al.*, 1998; Graybiel and Rauch, 2000; Aouizerate *et al.*, 2004b; Szechtman and Woody, 2004; Huey *et al.*, 2008; Szechtman *et al.*, 2014). The earliest evidence for this model of the pathophysiology of OCD emerged from PET studies that showed hyperactivation in the orbitofrontal cortex (OFC) and the caudate nucleus in patients with OCD; this hyperactivation resolved upon disappearance of OCD symptoms with therapy (Benkelfat *et al.*, 1990; Baxter, 1992; Baxter *et al.*, 1992; Swedo *et al.*, 1992). Subsequent brain imaging studies confirmed this finding and also observed hyperfunction of the basal ganglia and the limbic system in OCD (McGuire *et al.*, 1994; Rauch *et al.*, 1994; Saxena *et al.*, 1998; Adler *et al.*, 2000; Kim *et al.*, 2001; Mataix-Cols *et al.*, 2004; Phillips and Mataix-Cols, 2004; van den Heuvel *et al.*, 2004; Friedlander and Desrocher, 2006; Menzies *et al.*, 2008; Rotge *et al.*, 2008;

Rotge *et al.*, 2010). More recently, clinical trials with deep brain stimulation (DBS) directed at three sites – the subthalamic nucleus (Mallet *et al.*, 2002; Fontaine *et al.*, 2004), the anterior limbs of the internal capsules (Anderson and Ahmed, 2003; Nuttin *et al.*, 2003), and the nucleus accumbens (Sturm *et al.*, 2003; Denys *et al.*, 2010b; Huff *et al.*, 2010) – have shown therapeutic effects in some OCD patients (De Koning *et al.*, 2011; Kohl *et al.*, 2014). Although it is not well understood how DBS yields therapeutic effects, it may involve disruption or normalization of reverberating activity in basal ganglia loops (Tass *et al.*, 2003; Aouizerate *et al.*, 2004a; Haynes and Mallet, 2010; Bourne *et al.*, 2012; Figeo *et al.*, 2013), consistent with the proposed pathophysiology of OCD. Finally, a similar disruption or normalization of overactivity in the proposed loops may be expected from strategically placed lesions, and indeed severe cases of OCD may improve with psychosurgery, in particular, with anterior capsulotomy or cingulotomy (Kettl and Marks, 1986; Chiocca and Martuza, 1990; Jenike *et al.*, 1991; Baer *et al.*, 1995; Dougherty *et al.*, 2002).

It is not known what pathogenic mechanisms produce the overactivity. In the present study, we examine the development of compulsive checking in an animal model of OCD with the aim of identifying contributions of the nucleus accumbens core (NAc) toward the pathogenesis

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of OCD. It may be expected that the nucleus accumbens plays a role in the development of compulsive behavior because of the therapeutic efficacy of DBS in OCD patients as noted above. Consistent with the above clinical findings are results from animal studies where DBS of the nucleus accumbens and related sites was effective in attenuating compulsive checking (Winter *et al.*, 2008; Mundt *et al.*, 2009; Djodari-Irani *et al.*, 2011; Winter, 2012) in the same quinpirole sensitization rat model of compulsive checking as used in the present study. Finally, a contribution of the nucleus accumbens may be expected, given that a lesion of the NAc produces hyperactivity (Cardinal *et al.*, 2001; Dvorkin *et al.*, 2010; Tucci *et al.*, 2014b), raising the possibility that such hyperactive rats may develop compulsive checking faster with repeated injections of quinpirole.

One more set of considerations adds toward a rationale for examining how the nucleus accumbens may contribute toward the pathogenesis of compulsive checking. This set represents the confluence of three notions or lines of evidence. One line is the importance of the nucleus accumbens in motivation (Cools, 1980; Mogenson *et al.*, 1980; Wise, 2005; Salamone *et al.*, 2009; Bromberg-Martin *et al.*, 2010; Cools *et al.*, 2011; Da Cunha *et al.*, 2012). Another line concerns theories that cast OCD within a motivational framework (Szechtman *et al.*, 2004; Figeo *et al.*, 2011; Rodriguez-Romaguera *et al.*, 2012). The last line consists of arguments placing animal models of OCD within a motivational framework: the quinpirole sensitization rat model of compulsive checking (Szechtman *et al.*, 1998; Eilam and Szechtman, 2005; Szechtman and Eilam, 2005; Dvorkin *et al.*, 2010), the compulsive nest-building model in the rabbit (Hoffman and Morales, 2009; Hoffman, 2011; Hoffman and Rueda Morales, 2012), and the signal-attenuation rat model of compulsive lever pressing (Joel, 2006b; Albelda and Joel, 2012b), all showing findings consistent with a motivational perspective.

In the present study, we used the sensitization model of OCD, represented by compulsive checking that is induced by repeated treatment with the D2/D3 dopamine agonist quinpirole (Szechtman *et al.*, 1998, 1999; Eilam and Szechtman, 2005; Szechtman and Eilam, 2005). In the quinpirole sensitization rat model, compulsive checking is manifested by exaggerated preoccupation with one location in the environment, to which the animal returns repeatedly (for reviews of the model, see Szechtman *et al.*, 1998; Man *et al.*, 2004; Eilam and Szechtman, 2005; Szechtman and Eilam, 2005; Joel, 2006a; Korff and Harvey, 2006; Westenberg *et al.*, 2007; Boulougouris *et al.*, 2009b; Hoffman, 2011; Albelda and Joel, 2012a). The foundational claim for the model rests on experimental findings that the spatiotemporal structure of quinpirole-induced behavior matches the salient structural features of OCD checking in humans – an exaggerated preoccupation with the item(s) of concern, a ritual-like quality in motor performance, and environmental dependence for display of the behavior

(Reed, 1985; Szechtman *et al.*, 1998). A motivational framework for quinpirole-induced compulsive checking emerged subsequently. First, it became apparent that compulsive checking in the model may have a similar motivational basis as compulsive checking in humans. In humans, compulsive checking is seen as an exaggerated form of normal checking of one's well-being and security (Reed, 1985). In the model, too, a similar inference can be made because, as it turned out, the checking activity was directed toward a place with a plausible relationship with safety and security, namely, the 'home base' (Eilam and Golani, 1989), and in this respect, compulsive checking could be called an exaggerated form of normal checking in the rat, similar to the human condition. Second, encouraged by the motivational theory that OCD reflects a reduced satiety-like negative feedback (Szechtman *et al.*, 2004; Woody and Szechtman, 2005), we sought and documented a reduced negative feedback component between bouts of quinpirole-induced compulsive checking (Dvorkin *et al.*, 2006b). Finally, consistent with a motivational framework, we decomposed compulsive checking experimentally into three relatively independent functional components, all considerably exaggerated by quinpirole: (a) vigor of checking; (b) focus on checking; and (c) rest or 'satiety' after a bout of checking (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a). This decomposition exposed 'compulsive' behavior as highly motivated performance, but without apparent satiation (Dvorkin *et al.*, 2010). Thus, the quinpirole sensitization model of compulsive checking may be particularly suitable to examine the role of the NAc in the pathogenesis of OCD from a motivational perspective.

In the present study, contributions of NAc toward the development of quinpirole-induced compulsive checking were examined using a lesion approach. A lesion of the NAc was made and the induction of compulsive checking over the course of 10 quinpirole injections was monitored. A lesion-produced disruption in the development of compulsive checking to quinpirole should show which functional components are necessarily mediated by quinpirole-induced changes in the NAc and, by the same token, for which functional components changes induced by quinpirole outside the NAc are sufficient. Moreover, a lesion-produced disruption in the pathogenesis of compulsive checking may identify the functional components from which compulsive checking is assembled (Teitelbaum and Pellis, 1992; Teitelbaum, 2012).

## Methods

### Subjects

A total of 55 experimentally naive Long–Evans male rats (Charles River, St Constant, Quebec, Canada), weighing 250–300 g at the start of the experiment (~2 months of age), were used in the study. Animals were housed individually in a climate-controlled colony room on a 12 h light/dark cycle (06:00 h lights on, 18:00 h lights off).

Food and water were freely available. Upon arrival, rats were allowed to habituate to the animal facility for 7 days and were then handled for ~2–5 min each day for 5 days before surgery and in the week before the start of behavioral testing. Testing occurred during the light phase. Animals were housed and tested as approved by the Animal Research Ethics Board, McMaster University, in compliance with the Canadian Council on Animal Care guidelines.

### Drug treatments

Quinpirole hydrochloride (Sigma Aldrich, Oakville, Canada) was dissolved in 0.9% physiological saline and administered twice weekly at a dose of 0.5 mg/kg in a volume of 1 ml/kg through a subcutaneous injection under the nape of the neck, as in previous studies (Szechtman *et al.*, 1998; Dvorkin *et al.*, 2006b). Control animals were similarly injected with 1 ml/kg of 0.9% physiological saline. The elimination half-life of plasma quinpirole in the rat is about 9.5 h (Whitaker and Lindstrom, 1987).

### Apparatus

Animals were tested on a large open field (160 × 160 and 60 cm high table without walls) that was located in a noncolony experiment room illuminated by usual overhead fluorescent lights, as described previously (Dvorkin *et al.*, 2006b). The table was divided virtually into a grid of 25 rectangular places (locales), but no actual lines were marked on the table surface. Four small Plexiglas/glass boxes (~8 × 8 × 7.5 cm) were located at the same fixed location on the open field throughout the study: two at corners and two at places near the center of the open field. After each rat was tested, the table and objects were wiped clean with a diluted solution of an antibacterial cleaner (Lysol). Behavior on the open field was videotaped continuously by a camera affixed to the ceiling (providing a stationary top view of the entire open field and the rat in it). Videotapes were converted into MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder, Canopus Corporation, San Jose, California, USA) and these digitized videos were used to automatically track the trajectories of locomotion using EthoVision 3.1 (Noldus Information Technology BV, Wageningen, the Netherlands) software (Noldus *et al.*, 2001; Spink *et al.*, 2001).

### Surgery

The excitotoxin, *N*-methyl-D-aspartate (NMDA; Sigma Aldrich), was dissolved in PBS at a concentration of 12 mg/ml to produce neurotoxic lesions. For sham lesions, an equivalent volume of PBS was injected. Intracranial injections of NMDA and PBS were administered using a 10 µl noncoring Hamilton syringe (Hamilton Company, Reno, Nevada, USA) mounted to a motorized Ultra Micro Pump (World Precision Instruments, Sarasota, Florida, USA) that was attached to the arm of a Kopf Stereotaxic Apparatus (David Kopf

Instruments, Tujunga, California, USA). Vaporized isoflurane (Pharmaceutical Partners of Canada, Richmond Hill, Ontario, Canada) was used to anesthetize animals, and lidocaine hydrochloride (0.002 mg; Astra Zeneca, Mississauga, Ontario, Canada) was injected subcutaneously at the surgical site. The postoperative nonsteroidal anti-inflammatory analgesic Anafen (0.05 mg/kg; Merial, Baie d'Urfé, Québec, Canada) was administered subcutaneously 10 min before the end of surgery. Coordinates for the NAc lesion were as follows: AP, +1.2 mm from the bregma; ML, ±1.9 mm; and DV, –7.0 mm from the dura. At the injection site, 0.3 µl of the solution was injected bilaterally at a rate of 0.1 µl/min, and the needle was left in place for 5 min to allow for the neurotoxin to sufficiently diffuse away from the needle tip.

### Histology

After the final test, rats were euthanized using carbon dioxide. Brains were removed and flash frozen in –60°C methylbutane, placed on dry ice for 1 min, wrapped in an aluminum foil, and stored in a –80°C freezer until sectioning. Brains were mounted for sectioning using Tissue-Tek Optimum Cutting Temperature (Fisher Scientific, Toronto, Ontario Canada) compound and placed in a cryostat for 1 h to thaw to –20°C. The coronal plane was sectioned at 20 µm thickness, with every third section collected on a gelatin-coated slide and stored in a –35°C freezer until immunohistochemistry. The location and size of the lesions were visualized using neuronal nuclei (NeuN) protein immunohistochemistry: sections were stained using monoclonal mouse anti-NeuN (1 mg/ml; EMD Millipore, Billerica, Massachusetts, USA) as the primary antibody, followed by a biotinylated monoclonal anti-mouse IgG (0.5 mg/ml; Vector Laboratories, Burlington, Ontario, Canada) as the secondary antibody according to a described procedure (Jongen-Relo and Feldon, 2002). Following NeuN staining, each section was examined for the location and size of lesions using an Axioskope microscope and Axiovision 4.3 software system (Carl Zeiss Microimaging Inc., Thornwood, New York, USA). Lesion boundaries inside the region of interest (ROI) were demarcated, areas were computed, and expressed as a percentage of ROI area. To compute the ROI lesion area, brain sections at (or nearest to) the predetermined atlas plates (Paxinos and Watson, 1998) were taken (NAc: plates 11, 13, and 15) and the percentages of the ROI lesion at these plates were averaged to obtain the mean percent of ROI lesion. To be included for behavioral analysis, the minimum lesion size had to be 55% of the total ROI on the basis of the lesion criterion shown in Dvorkin *et al.* (2010).

### Data analysis

EthoVision 3.1 software was used to extract the time series of *x*, *y* coordinates of the rat from digitized video recordings (Dvorkin *et al.*, 2006b). To remove noise, digitized tracking data were preprocessed (by applying appropriate filters to smooth the *x*, *y* coordinates) (Hen *et al.*, 2004), and the coordinates obtained were divided into episodes of forward locomotion (called progression)

and episodes of small movements or immobility (called lingering), as described previously (Golani *et al.*, 1993; Drai *et al.*, 2000; Drai and Golani, 2001). The coordinate system was mapped onto the 25 open-field locales (places) (Szechtman *et al.*, 1998), and the frequency of visits and duration of stops in each locale were computed (the terms ‘visit’ and ‘stop’ are equivalent and are used interchangeably). Checking behavior was defined with reference to the most visited locale (labeled ‘key place’ or ‘key locale’; these terms are equivalent), which, in most instances, is also the locale with the longest total duration of stops (Eilam and Golani, 1989; Szechtman *et al.*, 1998). A visit to the key place is also referred to as a ‘check’ or ‘checking’, and the following sets of four measures of checking behavior were computed. (a) Frequency of checking: total number of visits to the key locale. (b) Length of check: total duration of stay at the key locale divided by the frequency of visits there; this measure is also an indirect index of ritual-like behavior as the appearance of motor rituals in quinpirole-treated rats is associated with a very short duration of stay in the key locale (Szechtman *et al.*, 1998; Ben Pazi *et al.*, 2001). (c) Recurrence time of checking: mean duration of return times to the key locale (‘return time’ is the interval from departure to next arrival at the locale). (d) Stops before returning to check: mean number of places visited between returns to the key locale. Compulsive checking behavior is identified by the presence of a significant difference between quinpirole-treated and saline-treated rats: all four measures need to differ from the controls to indicate ‘compulsive’ checking (Szechtman *et al.*, 1998), and hence the group of these four measures is termed ‘criteria measures’ for compulsive checking.

The criteria measures for compulsive checking were dissociated empirically in a lesion study (Dvorkin *et al.*, 2010). Specifically, a lesion to the NAc altered the amount of checking behavior (as indexed by the frequency of checking and length of check), whereas a lesion to the OFC affected the delay between checks of the key locale (as indexed by time to return to check and number of stops before returning to check). This pattern of results suggested that the functional roles of the NAc and OFC in checking behavior are to control the vigor of motor performance and the focus on goal-directed activity, respectively (Dvorkin *et al.*, 2010). Accordingly, we consider vigor and focus as two relatively independent components of checking behavior, with the vigor of checking indexed jointly by frequency of checking and length of check and the focus on checking indexed jointly by time to return to check and number of stops before returning to check.

In addition to the above criteria measures, we also evaluated ‘time to next checking bout’ (Dvorkin *et al.*, 2006b). This measure is markedly reduced in quinpirole-sensitized rats and has been proposed to index the third constitutive component of compulsive checking behavior

– ‘satiety’ or rest after checking (Dvorkin *et al.*, 2010). It was reasoned (Dvorkin *et al.*, 2010) that in the animal model, the reduced ‘satiety’ or ‘rest’ after a bout of checking corresponds to notions that OCD reflects failure in ‘sense of task completion’ (Pitman, 1989), ‘just right feeling’ (Leckman *et al.*, 1994; Wahl *et al.*, 2008), ‘feeling of incompleteness’ (Rasmussen and Eisen, 1992; Summerfeldt, 2004; Zor *et al.*, 2011), or ‘feeling of knowing’ (Szechtman *et al.*, 2004; Hinds *et al.*, 2012).

The computation of checking bouts is detailed in Dvorkin *et al.* (2006b). Briefly, the method follows the logic used to identify the clustering of a bout of eating behavior into a ‘meal’ and the time between meals into a period of postingestion satiety (Tolkamp *et al.*, 1998; Tolkamp and Kyriazakis, 1999). A bout of behavior, according to these authors, is defined on the basis of the distribution of time intervals between behavioral events (interevent intervals). This distribution is examined to locate and extract a time-point that will produce a natural split between clusters of interevent intervals. Specifically, the identified time-point will separate the time intervals into a class of (relatively long) intervals that are between the bouts of behavior (interbout intervals) and a class of (relatively shorter) intervals that belong within a bout of behavior (intraabout intervals) (Tolkamp *et al.*, 1998; Tolkamp and Kyriazakis, 1999). This principle was used in an algorithm developed to identify bouts of checking behavior (Dvorkin *et al.*, 2006b) and extract ‘time to next checking bout’. A rat may complete a bout of checking but not start the next bout during the session and hence the number of rats used for analysis of ‘time to next checking bout’ is generally smaller than for analysis of criteria measures for compulsive checking. Generally, saline-treated rats have one to two bouts of checking behavior in a session whereas quinpirole-treated rats usually perform two or more bouts (Dvorkin *et al.*, 2010). Following the modification in Tucci *et al.* (2013), even if more than one bout of checking was performed, only the first ‘time to next checking bout’ is used for statistical analysis.

### Design and procedure

The study involved a 2 × 2 fully crossed factorial design with two between-group factors: Lesion (sham lesion vs. NAc lesion) and the Drug (saline vs. quinpirole). Animals were assigned to treatment groups at random.

Behavioral testing began 2 weeks after surgery, together with the start of quinpirole or saline injections. For all tests, the same procedure was followed: animals were weighed, transported in their home cage to an adjoining noncolony experimental testing room, and administered the appropriate injection. Immediately afterwards, the rat was placed into the open field for 55 min and its behavior was videotaped for offline analysis. Each rat was subjected to two open-field tests per week, and was run for 10 trials on the same assigned days of the week (Monday/

Thursday or Tuesday/Friday), at approximately the same time of day, and by the same experimenter. Each experimenter was assigned a balanced number of rats from every experimental group.

### Statistical analysis

To assess the development of checking behavior across injections 1–10, regression estimates for each dependent variable were computed for each rat. The individual slopes and intercepts obtained were then analyzed statistically using a Lesion (sham vs. NAc lesion) by Drug (saline vs. quinpirole) analysis of variance (ANOVA); a significant Lesion  $\times$  Drug interaction was followed by the Duncan Multiple Range test to identify between-group differences. We chose the regression estimates approach over repeated-measure ANOVAs to analyze each dependent measure because the regression approach includes more rats in the analysis. In particular, the sporadic absence of compulsive checking during the course of treatment or a random technical malfunction does not eliminate the individual rat from analysis of regression parameters, but impacts the number of data points in a repeated-measures ANOVA. To correct for skew in the data, the variables length of check and time to next checking bout were log transformed for statistical analysis. The chosen level of significance was  $P$  less than 0.05. Calculations were carried out using IBM SPSS Statistics 20.0.

## Results

### Histology

As in our other NAc lesion studies (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a), animals that had at least a 55% lesion to the NAc were included in the study. The final number of rats in each group was as follows: lesion-saline,  $N=10$ ; lesion-quinpirole,  $N=14$ ; sham-saline,  $N=11$ ; and sham-quinpirole,  $N=10$ . The mean size of the cell-body lesion was  $72\pm 3\%$  of NAc in the lesion-saline group and  $74\pm 1\%$  in the lesion-quinpirole group, with no measurable cell damage in the sham groups. Cell body destruction was comparable to our other studies and well localized within the accumbens core subregion, with minimal encroachment to the accumbens shell subregion or the ventral pallidum (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a).

### Profile of induction of compulsive checking by quinpirole in sham lesion rats

To establish the framework within which to assess how a lesion of the NAc impacts the development of compulsive checking to quinpirole, we first examine the sham groups and consider how measures of compulsive checking change during the course of quinpirole treatment compared with injections of saline. This comparison of the sham-quinpirole with the sham-saline group also serves to verify that control animals developed compulsive checking, as expected.

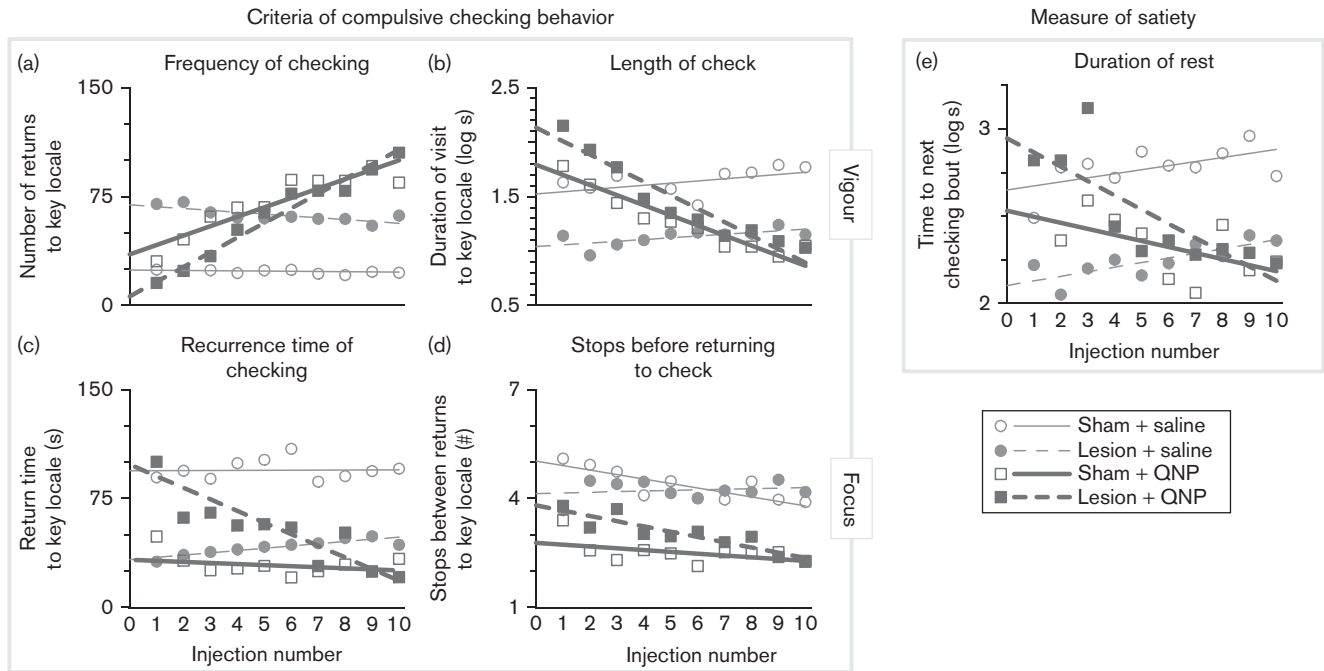
Figure 1 (left panel) shows the criteria measures that define the presence of compulsive checking – frequency of checking, length of check, recurrence time of checking, and stops before returning to check. The graph shows the profile of these measures during injections 1–10 for each group: sham-saline and sham-quinpirole rats are represented, respectively, by the open circles and the open squares; the calculated regression lines for the saline and quinpirole sham groups are indicated by the solid thin line and the solid thick line, respectively. Inspection of these regression lines suggests that chronic treatment with quinpirole produced two distinct effects on the criteria measures of compulsive checking: for frequency of checking and length of check (Fig. 1a and b), repeated injections of quinpirole altered the slope, but not the intercept of the regression line, compared with the sham-saline group. For recurrence time of checking and stops before returning to check (Fig. 1c and d), quinpirole produced a shift in the intercept of the regression line without an effect on the slope, compared with the sham-saline group. The statistical analysis shown in Table 1 supports these observations.

As noted in the Materials and methods section, frequency of checking and length of check are variables that index the vigor in the motor performance of checking whereas recurrence time of checking and stops before returning to check are variables related to the focus on the task of checking. Accordingly, the finding of altered slope, but not intercept for measures of vigor indicate that only the vigor of checking sensitizes with repeated injections of quinpirole (Table 1). In contrast, the findings of altered intercept but not slope for measures of focus indicate that quinpirole increases focus acutely, and this acute effect persists unabated throughout the course of chronic quinpirole treatment (Table 1).

Figure 1e shows a similar regression analysis for the variable *duration of rest* as indexed by ‘time to the next checking bout’. This variable is suggested to constitute a component of compulsive checking related to ‘satiety’ or negative feedback engendered by completion of the task of checking (Dvorkin *et al.*, 2006b; Dvorkin *et al.*, 2010). Inspection of Fig. 1e and the results of statistical analysis presented in Table 1 show that this variable changed across quinpirole injections in a similar manner as observed with length of check, namely, a decrease in their durations with repeated injections of quinpirole (Table 1).

In all, the present findings for the induction of compulsive checking in sham lesion rats replicate the pattern of results for induction of compulsive checking observed in intact rats (Tucci *et al.*, 2014b) as well as the pattern of results found earlier using a somewhat different method of statistical analysis (Dvorkin *et al.*, 2006b).

Fig. 1



Effects of chronic treatment with quinpirole (QNP) on the criteria measures for compulsive checking (a–d) and on the measure of postchecking satiety (e). See Table 1 for parameters of the indicated regression lines. Each symbol is the mean value for the indicated dependent variable at the indicated injection obtained from all rats showing a value. Note that the parameters of the indicated regression lines were not obtained using these actual means, but were computed from the regression parameters of each individual rat as described in the Materials and methods. Open circles, sham-control group injected repeatedly with saline (sham-saline group); open squares, sham group injected repeatedly with QNP (sham-QNP group); solid circles, nucleus accumbens core (NAc) lesion group injected repeatedly with saline (lesion-saline group); and solid squares, NAc lesion group injected repeatedly with QNP (lesion-QNP group). The corresponding regression lines are as follows: thin solid line, sham-saline group; thin dashed line, lesion-saline group; thick solid line, sham-QNP group; and thick dashed line, lesion-QNP group.

### Effects of nucleus accumbens core lesion on induction of compulsive checking

#### Effects of nucleus accumbens core lesion on saline-treated rats

As shown in Fig. 1 and Table 1, compared with sham-saline controls, a lesion of the NAc produced a significant shift in the intercept of the regression line for four of the five dependent measures. Specifically, the NAc lesion increased markedly the vigor of checking in saline-treated rats, as evidenced by a statistically significant increase in the intercept for frequency of checking and a decrease in the intercept for length of check. Moreover, the NAc lesion produced a significant decrease in the intercept for one measure of focus – recurrence time of checking – but had no effect on the intercept for the other measure of focus on checking, namely, stops before returning to check. Finally, compared with sham-saline controls, the NAc lesion reduced satiety after a bout of checking, as evidenced by a statistically significant reduction in the intercept for duration of rest (Table 1).

In contrast to effects on intercept, the NAc lesion exerted no significant effect for any variable on the slope of the regression line compared with sham-saline controls

(Table 1). This suggests that relative to sham-saline controls, the effects of the NAc lesion were relatively stable during the 5 weeks of testing.

In all, the NAc lesion pushed performance toward a compulsive checking profile in that lesion-saline rats performed on four of five measures of checking behavior at a level that was comparable to sham-quinpirole rats after quinpirole injections (Fig. 1). In particular, visual inspection of the regression lines in Fig. 1 suggests that performance on measures of vigor (Fig. 1a and b) and 'satiety' (Fig. 1e) in lesion-saline rats matched the performance of sham-quinpirole rats after five to seven quinpirole injections. Furthermore, performance on recurrence time of checking (Fig. 1c) in lesion-saline rats was equivalent at the start of testing to performance induced by quinpirole in sham-quinpirole rats. By the same token, it is equally important to note that lesion-saline rats cannot be deemed to show compulsive checking behavior. This is because one of the required criteria measures for establishing the presence of compulsive checking (stops before returning to check) did not differ between lesion-saline and sham-saline controls.

**Table 1 Parameters of regression lines shown in Fig. 1 for compulsive checking and in Fig. 3 for measures of locomotion**

Compulsive checking and locomotion measures	Regression parameters <sup>a</sup>	Group <sup>b</sup>				Drug effect <sup>c</sup>			Lesion effect <sup>c</sup>			Drug × lesion interaction <sup>c</sup>		
		Sham-sal	Lesion-sal	Sham-QNP	Lesion-QNP	F(1,41)	P	$\eta_p^2$	F(1,41)	P	$\eta_p^2$	F(1,41)	P	$\eta_p^2$
Frequency of checking	Intercept	<b>24.37 ± 6.12</b>	<b>69.29 ± 6.42<sup>d</sup></b>	<b>35.19 ± 6.42<sup>e</sup></b>	<b>6.31 ± 5.43<sup>def</sup></b>	18.210	0.000	0.308	1.722	0.197	0.040	36.473	0.000	0.471
	Slope	-0.14 ± 0.95	-1.27 ± 0.99	<b>6.47 ± 0.99<sup>de</sup></b>	<b>10.10 ± 0.84<sup>def</sup></b>	90.285	0.000	0.688	1.749	0.193	0.041	6.334	0.016	0.134
Length of check (log s)	Intercept	<b>1.52 ± 0.14</b>	<b>1.04 ± 0.15<sup>d</sup></b>	<b>1.79 ± 0.15<sup>e</sup></b>	<b>2.13 ± 0.13<sup>de</sup></b>	22.883	0.000	0.358	0.251	0.619	0.006	8.347	0.006	0.169
	Slope	0.02 ± 0.02	0.02 ± 0.02	<b>-0.09 ± 0.02<sup>de</sup></b>	<b>-0.12 ± 0.02<sup>de</sup></b>	51.775	0.000	0.558	0.996	0.324	0.024	0.596	0.444	0.014
Recurrence time of checking (s)	Intercept	<b>94.18 ± 14.17</b>	<b>32.97 ± 14.86<sup>d</sup></b>	<b>32.91 ± 14.86<sup>d</sup></b>	<b>98.41 ± 12.56<sup>ef</sup></b>	0.022	0.884	0.001	0.023	0.881	0.001	20.061	0.000	0.329
	Slope	0.05 ± 1.65	1.55 ± 1.73	-0.74 ± 1.73	<b>-8.00 ± 1.46<sup>def</sup></b>	9.866	0.003	0.194	3.066	0.087	0.070	7.081	0.011	0.147
Stops before returning to check (#)	Intercept	<b>5.04 ± 0.46</b>	<b>4.14 ± 0.49</b>	<b>2.78 ± 0.49<sup>d</sup></b>	<b>3.81 ± 0.41</b>	7.827	0.008	0.160	0.023	0.881	0.001	4.367	0.043	0.096
	Slope	-0.12 ± 0.06	0.02 ± 0.06	-0.05 ± 0.06	<b>-0.15 ± 0.05</b>	0.579	0.451	0.014	0.148	0.703	0.004	4.267	0.045	0.094
Duration of rest (log s)	Intercept	<b>2.72 ± 0.24</b>	<b>1.83 ± 0.24<sup>d</sup></b>	<b>2.54 ± 0.24<sup>e</sup></b>	<b>2.70 ± 0.21<sup>e</sup></b>	2.228	0.144	0.054	2.458	0.125	0.059	5.167	0.029	0.117
	Slope	0.02 ± 0.04	0.11 ± 0.04	-0.04 ± 0.04 <sup>de</sup>	-0.06 ± 0.04 <sup>de</sup>	6.891	0.012	0.150	0.745	0.393	0.019	2.039	0.161	0.050
Distance (m)	Intercept	<b>108.93 ± 22.65</b>	<b>314.01 ± 23.76<sup>d</sup></b>	<b>78.11 ± 23.76<sup>e</sup></b>	<b>15.14 ± 20.08<sup>de</sup></b>	53.145	0.000	0.565	9.874	0.003	0.194	35.128	0.000	0.461
	Slope	-1.97 ± 3.81	-1.10 ± 4.00	<b>34.82 ± 3.99<sup>de</sup></b>	<b>38.53 ± 3.38<sup>de</sup></b>	100.983	0.000	0.711	0.363	0.550	0.009	0.140	0.711	0.003
2SDE	Intercept	<b>6.12 ± 0.37</b>	<b>5.68 ± 0.39</b>	<b>2.58 ± 0.39<sup>de</sup></b>	<b>3.05 ± 0.33<sup>de</sup></b>	68.609	0.000	0.626	0.003	0.957	0.000	1.497	0.228	0.035
	Slope	-0.03 ± 0.04	<b>0.08 ± 0.04</b>	0.06 ± 0.04	0.03 ± 0.03	0.136	0.714	0.003	1.178	0.284	0.028	3.713	0.061	0.083
Path stereotypy (ratio)	Intercept	<b>2.31 ± 0.20</b>	<b>2.89 ± 0.21</b>	<b>2.57 ± 0.21</b>	<b>2.03 ± 0.18<sup>e</sup></b>	2.313	0.136	0.053	0.012	0.913	0.000	7.784	0.008	0.160
	Slope	<b>0.03 ± 0.04</b>	<b>0.04 ± 0.05</b>	<b>0.23 ± 0.05<sup>de</sup></b>	<b>0.29 ± 0.04<sup>de</sup></b>	26.760	0.000	0.395	0.470	0.497	0.011	0.290	0.593	0.007

2SDE, 2 standard deviational ellipse.

<sup>a</sup>Estimates of intercept and slope are means (and SEM) of individual rat regression parameters fitted to the dependent variable data across injections 1–10. Values in bold font are significantly different from 0.

<sup>b</sup>Sham-sal refers to the control group that received a sham lesion and was injected chronically with saline; the lesion-sal group refers to rats that had a nucleus accumbens core (NAc) lesion and were injected chronically with saline; the sham-QNP group are sham lesion rats treated chronically with quinpirole (0.5 mg/kg); and the lesion-QNP group are NAc lesion rats treated chronically with quinpirole (0.5 mg/kg).

<sup>c</sup>Groups were evaluated using a drug-by-lesion analysis of variance and the *F* values obtained, statistical significance, and partial eta squared values ( $\eta_p^2$ ) are indicated. Following a significant drug × lesion interaction, the Duncan multiple range test was used for post-hoc comparisons. Significant differences (*P* < 0.05) are indicated by a letter superscript.

<sup>d</sup>Versus sham-sal.

<sup>e</sup>Versus lesion-sal.

<sup>f</sup>Versus sham-QNP.

**Effects of nucleus accumbens core lesion on quinpirole-treated rats**

Inspection of Fig. 1 shows clearly that the NAc lesion did not preclude the induction of compulsive checking by chronic quinpirole treatment as at the end of quinpirole treatment, all measures of compulsive checking were conspicuously similar in the sham-quinpirole and lesion-quinpirole groups. Although the NAc lesion did not stop the induction of compulsive checking, the time course of this induction for two criteria measures was affected by the lesion. Specifically, both the intercept and the slope of the regression lines for frequency of checking and recurrence time of checking differed significantly between lesion-quinpirole and sham-quinpirole groups (Table 1). Inspection of those regression lines in Fig. 1 suggests that for these two compulsive checking criteria measures, the NAc lesion attenuated and/or retarded the impact of the first few quinpirole injections on the induction of compulsive checking.

**Effects of nucleus accumbens core lesion on routes of travel**

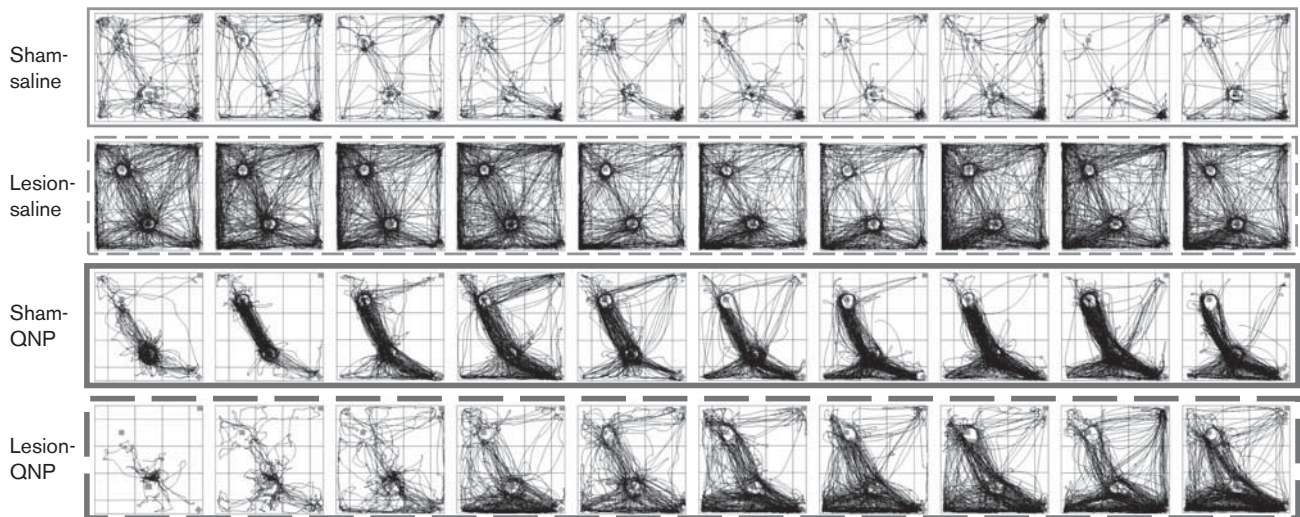
**Visual inspection of routes of travel**

Figure 2 shows the trajectories of locomotion shown by a rat from each group during the course of 10 treatments with saline or quinpirole. The chosen rats were selected because each one shows the key features in their group of change across injections. Quantitative analyses for routes of travel are presented in Fig. 3 and involve variables shown previously to show a specific profile that accompanies quinpirole-induced compulsive checking (Eilam

*et al.*, 1989; Szechtman *et al.*, 1994; Dvorkin *et al.*, 2006a, 2006b, 2010). In particular, routes of travel by quinpirole rats with compulsive checking are characterized by the following: (a) elevated amount of locomotion, as measured by the distance traveled; (b) shrinkage of explored space, as measured by 2 standard deviational ellipse (2SDE); and (c) restriction of locomotion to repeated travel along a few routes only, as measured by path stereotypy. These changes in each variable are clearly evident from a visual comparison of routes of travel shown by the sham-saline and the sham-quinpirole rat on injection 10 (Fig. 2, top and third rows, respectively). More locomotion (and hence a higher value for the distance traveled) is evidenced by greater density of trajectories in the sham-quinpirole rat compared with the saline control. Shrinkage of explored space in the sham-quinpirole rat (and hence a lower value for 2SDE) is evidenced by paths being distributed over a relatively narrow region of the open field versus paths of the sham-saline rat being dispersed over a large area bounded by arena borders. Finally, more repetitions of travel along a few routes (and hence a higher value for path stereotypy) are evidenced by few yet thick trajectories in the sham-quinpirole rat compared with many and thin trajectories of locomotion in the sham-saline rat.

As is evident in Fig. 2 (second row), the NAc lesion rat injected with saline was hyperactive and showed a high level of locomotion much throughout the 10 test sessions; it locomoted over the entire arena up to the borders of the open field and yet, it did not develop conspicuous routes of travel. In essence, the lesion-saline rat did not

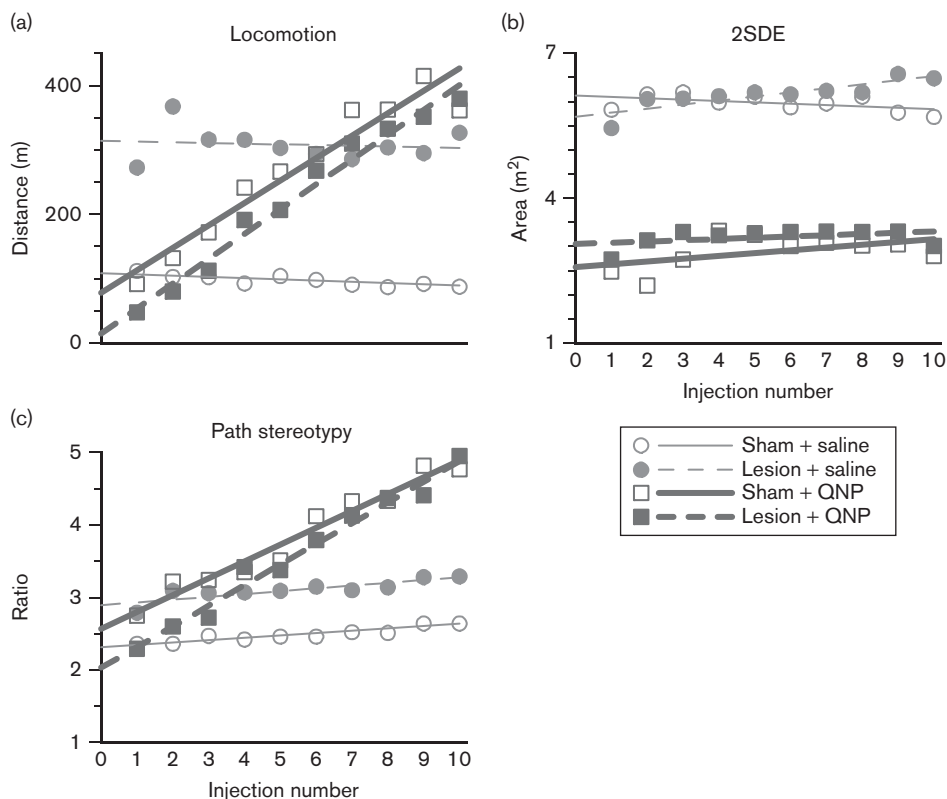
**Fig. 2**



Effects of chronic treatment with quinpirole (QNP) on the routes of travel in sham and nucleus accumbens core (NAc) lesion rats. Routes of travel are shown as path plots for a representative rat with a sham lesion (first and third rows) or NAc lesion (second and fourth rows) that was treated either with saline (top two rows) or QNP (bottom two rows). Locomotor trajectories during the entire 55 min session for injections one to 10 are shown. Each line represents a trajectory of locomotion and the density of trajectory lines corresponds to the amount of locomotion. Gray squares indicate locations of the four objects in the open field.



Fig. 3



Effects of chronic treatment with quinpirole (QNP) on the distance traveled (a), 2 standard deviational ellipse (2SDE) (b), and path stereotypy (c) in sham and nucleus accumbens core (NAc) lesion rats. See Table 1 for parameters of the indicated regression lines. Open circles, sham-control group injected repeatedly with saline (sham-saline group); open squares, sham group injected repeatedly with QNP (sham-QNP group); solid circles, NAc lesion group injected repeatedly with saline (lesion-saline group); and solid squares, NAc lesion group injected repeatedly with QNP (lesion-QNP group). The corresponding regression lines are as follows: thin solid line, sham-saline group; thin dashed line, lesion-saline group; thick solid line, sham-QNP group; and thick dashed line, lesion-QNP group.

show the routes of travel typical of quinpirole rats showing compulsive checking. In contrast, as shown in Fig. 2 (bottom row), the lesion-quinpirole rat did develop the typical routes of travel, although their emergence appears delayed compared with the sham-quinpirole rat (Fig. 2, third row). These observations are supported by quantitative measures shown in Fig. 3 and statistical analyses presented in Table 1 and summarized below.

#### Analysis of measures of routes of travel

Comparison of the regression lines in Fig. 3 for sham-quinpirole and sham-saline groups shows how changes in measures of routes of travel accompany the typical profile for the development of quinpirole-induced compulsive checking. Statistical analyses of the parameters of the regression lines are presented in Table 1 and indicate the following properties of the profile for measures of routes of travel. The amount of locomotion sensitizes with repeated injections of quinpirole, as is evident by a significant increase in the slope of the regression line for sham-quinpirole versus sham-saline rats, and no change in intercept (Table 1). The spatial extent of explored

space, as indexed by 2SDE, is constricted by acute quinpirole and remains so throughout testing, as is evident from a significant lowering of the intercept of the regression line for sham-quinpirole versus sham-saline rats, and the flat slope across injections (Table 1). Finally, repeated travel along a few routes, as indexed by path stereotypy, increases with repeated injections of quinpirole, as is evident from a significantly steeper slope of the regression line for sham-quinpirole versus sham-saline rats, and no change in intercept (Table 1).

Regression lines for locomotion, 2SDE, and path stereotypy in lesion-saline rats (Fig. 3), suggest that the NAc lesion did not induce the profile of sham-quinpirole rats. Indeed, the NAc lesion raised the intercept for locomotion and did not alter the slope of the regression line in the lesion-saline group compared with sham-saline controls (Table 1). The significant shift in intercept indicates that the NAc lesion induced hyperlocomotion in lesion-saline rats. The increase in the amount of locomotion was considerable – equivalent to that of sham-quinpirole rats that were administered six injections of quinpirole

(Fig. 3). However, lesion-saline rats continued at this same (elevated) level during the 10 test sessions, as evidenced by a flat slope of the regression line and the absence of a lesion effect on slope compared with sham-saline controls (Table 1). Moreover, the NAc lesion exerted no statistically significant effects on intercept and slope for 2SDE and path stereotypy (Table 1). This indicates that unlike quinpirole in sham-quinpirole animals, the NAc lesion did not shrink the explored space in lesion-saline rats and neither did it confine routes of travel to only a few paths. Nevertheless, there was a trend for the path stereotypy intercept to be elevated, suggesting a small increase in repeated travel along some routes in lesion-saline rats (Fig. 3).

Regression lines for locomotion, 2SDE, and path stereotypy in lesion-quinpirole rats (Fig. 3) support the observation from Fig. 2 (bottom row) that, even in rats with a NAc lesion, quinpirole treatment induces routes of travel characteristic of quinpirole rats with compulsive checking. Indeed, as shown in Table 1, lesion-quinpirole and sham-quinpirole rats did not differ on any parameter of the regression line for locomotion, 2SDE, or path stereotypy.

## Discussion

Compulsive checking behavior induced by the dopamine agonist quinpirole is not present upon the first injection of the drug – it develops as a function of the number of drug treatments and is generally fully formed after six to 10 administrations of quinpirole (Dvorkin *et al.*, 2006b). By analyzing how a lesion of the NAc impacts the development of this compulsive checking, the present study provides evidence for three relatively novel conclusions regarding the pathogenesis of this model OCD behavior. First, our results indicate that the NAc can modulate the time course of pathogenesis, but is not necessary for compulsive checking to develop. Second, the pathogenesis of compulsive checking involves two distinct neurochemical mechanisms – activation of dopamine D2/D3 receptors without concurrent stimulation of D1-like receptors and long-term plastic changes related to quinpirole-induced sensitization. The former mechanism is important for the focus component of compulsive checking whereas the latter mechanism is important for the vigor and satiety components. Third, our results show that a state of prolonged hyperactivity does not yield compulsive checking behavior, indicating that hyperactivity by itself is not sufficient for the pathogenesis of compulsive checking. Together, these findings strengthen the evidence that compulsive checking is not a unitary phenomenon, but constitutes separate functional components. Below, we elaborate on the evidence for the above conclusions and suggest that the NAc may serve as a hub that coordinates the activation of the various components. We also suggest the

usefulness of a motivational framework for compulsive checking and OCD more generally.

### Pathogenesis of compulsive checking delayed by nucleus accumbens core lesion but not stopped

As shown in previous studies (Dvorkin *et al.*, 2006b; Tucci *et al.*, 2014b), the present results constitute a third independent replication showing that repeated injections of quinpirole produce different effect patterns for different components of compulsive checking. Specifically, the vigor component starts off at the level of saline controls and builds up slowly with each successive quinpirole injection until becoming markedly different from saline controls (Fig. 1a and b). In contrast, development of the focus component is quick as focus is maximally enhanced after one or two quinpirole injections (Fig. 1c and d). Finally, the third component of compulsive checking, satiety after a bout of checking, wanes with successive injections of quinpirole until the duration of satiety decreases to a level markedly shorter than that in saline controls (Fig. 1e). The novel contribution of the present study comes from analysis of how the above developmental time course was impacted by the NAc lesion. The results indicated that a lesion of the NAc altered the profiles for frequency of checking and recurrence time of checking, as evidenced by parameters of regression lines for these two measures being significantly different between lesion-quinpirole and sham-quinpirole groups. These effects of the lesion were transitory because only the first few injections of quinpirole had a reduced impact on checking behavior – by the end of the chronic treatment, there was no difference between lesion-quinpirole and sham-quinpirole groups in their compulsive checking behavior.

The finding that a NAc lesion does not prevent induction of compulsive checking provides strong evidence that an intact NAc is not necessary for the pathogenesis of compulsive behavior, and by implication that changes produced by quinpirole in brain regions outside the NAc are sufficient for compulsive checking to develop. The latter claim assumes that the small amount of NAc tissue that was spared from lesion (about 25% of NAc) does not subsume the function of an entire intact NAc. This assumption is reasonable, given that the effects of the lesion were long-lasting in lesion-saline rats but short-lived in lesion-quinpirole animals. If the latter effect was because of complete compensation by the spared NAc, this would imply that quinpirole induces very rapid recovery of damaged tissue, and such a proposition seems unlikely. A previous study (Dvorkin *et al.*, 2010) provides additional evidence that changes outside the NAc are sufficient for quinpirole-induced compulsive checking. In that study, a lesion of the NAc was performed at the end of chronic treatment with quinpirole, when compulsive checking was already induced. In those animals, too, there was little effect of the NAc lesion on

quinpirole-induced compulsive checking. Thus, not only is an intact NAc unnecessary to induce compulsive checking but also a NAc lesion does not disrupt compulsive checking established before surgery.

The finding that quinpirole regression lines for frequency of checking and recurrence time of checking are altered by NAc lesion suggests a modulatory role of NAc on these measures. Inspection of the lesion-quinpirole regression lines suggests decreased impact from the first few administrations of quinpirole (Fig. 1a and c). Hence, the initially reduced pathogenesis of compulsive checking suggests that NAc normally facilitates the effects of quinpirole at the start of treatment and accordingly, with that influence removed, more quinpirole outside NAc is required to initiate pathogenesis.

In all, our lesion findings provide compelling evidence that the NAc can modulate the time course of pathogenesis of compulsive checking and, yet, quinpirole-induced compulsive checking can develop even without a normally functioning NAc.

### **Distinct neurochemical mechanisms for components of compulsive checking**

#### **Focus component**

In addition to the well-recognized role that dopamine systems play in reward and motivational processes (Wise, 2013), dopamine is increasingly being recognized for its role in mediating switching between tasks (Cools, 1980; Oades, 1985; van den Bos and Cools, 1989) and more generally in processes related to cognitive flexibility (Floresco, 2013; Klanker *et al.*, 2013; Hatalova *et al.*, 2014). In the quinpirole sensitization model of OCD, one of the identified constitutive components of compulsive checking is focus on the goal-directed activity of checking the 'home base', with the focus being considerably enhanced by quinpirole (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a). The 'focus' component is probably an aspect of the processes subsumed under 'cognitive flexibility', where a quinpirole-induced exaggerated focus would be manifested as a reduction in cognitive flexibility. Indeed, tests of cognitive flexibility have found that treatment with quinpirole reduces flexibility (Kurylo, 2004; Boulougouris *et al.*, 2009a; Eagle *et al.*, 2014; Hatalova *et al.*, 2014) and that reduced cognitive flexibility during task-switching is present in individuals with OCD (Chamberlain *et al.*, 2006; Chamberlain *et al.*, 2007; Gu *et al.*, 2008).

In the quinpirole sensitization model, focus is identified by two concurrent measures – recurrence time of checking and stops before returning to check – and both need to differ from saline controls for the claim of enhanced focus (Dvorkin *et al.*, 2010). Shrinkage of explored space, as indexed by 2SDE, shows the same profile of response to quinpirole as those measures, and hence 2SDE may be another indicator of enhanced focus.

As replicated here, enhanced focus is mediated by mere activation of D2/D3 receptors as it is present in sham-quinpirole rats from the first quinpirole injection onwards, with no evidence of sensitization or tolerance (Dvorkin *et al.*, 2006b; Tucci *et al.*, 2014b). As shown in the present study, because quinpirole-induced exaggerated focus was present in rats with a lesion to the NAc, D2/D3 receptors outside NAc are sufficient to mediate enhanced focus. The present study does not identify their neuroanatomical location, but the literature on cognitive flexibility points to the prefrontal cortex and striatum as likely candidate regions (Klanker *et al.*, 2013); another candidate site is the OFC as this region had been linked to the focus component in a lesion study (Dvorkin *et al.*, 2010).

The results from lesion-saline rats provide additional detail on the neurochemical mechanism mediating enhanced focus. In particular, they suggest that the focus component of compulsive checking requires persistent activation of D2/D3 receptors, but without concurrent stimulation of D1-like receptors. This hypothesis follows from the finding that enhanced focus did not develop in lesion-saline rats, despite repeated testing and continued hyperactivity. The fact that enhanced focus did not emerge in lesion-saline rats suggests that the exaggerated focus produced by quinpirole in normal rats reflects more than mere dopamine release. Our test situation – exposure of the animal to a large open field – would be expected to spontaneously activate dopamine systems (Feenstra *et al.*, 1995; Feenstra and Botterblom, 1996; Legault and Wise, 2001) and yet there was no decrease in stops before returning to check, and consequently, enhanced focus did not emerge in lesion-saline rats. We hypothesize that exaggerated focus requires persistent stimulation of D2/D3 receptors without concurrent activation of D1-like receptors, a condition not normally encountered with dopamine release. However, such a condition does occur under quinpirole because this drug is not only an agonist of D2/D3 receptors but also inhibits dopamine release (Imperato *et al.*, 1988; Koeltzow *et al.*, 2003; Anzalone *et al.*, 2012), creating a state of high postsynaptic D2/D3 activation with low dopamine release, and hence minimal or low stimulation of D1-like receptors.

#### **Vigor and satiety components**

Although even a single injection of quinpirole can enhance focus in normal rats, this is not the case for enhanced vigor. The vigor component becomes markedly different from saline controls only after repeated injections of quinpirole as the intensity of checking performance builds up slowly with each successive quinpirole injection. In other words, the vigor component shows sensitization to quinpirole, in parallel to the development of locomotor sensitization to quinpirole. The development of the satiety component also shows sensitization to quinpirole. As shown here, this sensitization process is still present after an NAc lesion,

indicating that the behaviorally relevant modifications from quinpirole-induced sensitization stem from stimulation of D2/D3 receptors outside NAc.

Although the present study does not identify their neuroanatomical location, it does suggest that sufficient D2/D3 receptors for sensitization of vigor and satiety are probably downstream from NAc. This suggestion emerges from the following observations. First, as found previously, a NAc lesion enhanced vigor and satiety in saline-treated rats, indicating that the circuit mediating vigor and satiety includes the NAc itself, exerting inhibitory control over these components (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a). Second, the NAc lesion altered the development of quinpirole sensitization (at least as it related to frequency of checking; Fig. 1a), indicating less control over the sensitization process without NAc. Finally, acute quinpirole inhibited the lesion-induced elevated vigor and satiety (compare the performance of lesion-quinpirole and lesion-saline rats on injection 1; Fig. 1), suggesting that quinpirole acted on the NAc disinhibited connections. Efferents from the NAc project to the dorsolateral ventral pallidum, medial entopeduncular nucleus region, medial subthalamic nucleus region, and substantia nigra pars reticulata and compacta (Zahm, 2000). Considering that most of these structures receive dopaminergic input (Gurevich and Joyce, 1999), the critical D2/D3 receptors for sensitization of vigor and satiety may be located at one or more of these sites.

In all, although enhanced focus involves acute stimulation of D2/D3 receptors without concurrent stimulation of D1-like receptors, the neurochemical mechanisms mediating vigor and satiety components of compulsive checking involve sensitization to quinpirole and hence some long-term plastic changes produced by chronic stimulation of D2/D3 receptors with quinpirole.

#### **Hyperactivity not sufficient for compulsive checking**

Lesions of the NAc produce hyperactivity (Cardinal *et al.*, 2001) and this is also evident in the present study, as indexed by higher locomotion in lesion-saline animals (Fig. 3a). In a previous study (Dvorkin *et al.*, 2010), we argued that because a lesion of the NAc induced hyperactivity, but not compulsive checking, this showed that mere locomotor hyperactivity does not result in compulsive checking behavior. The present study extends this argument and shows that this lack of compulsive checking in hyperactive NAc lesion rats is not due to insufficient opportunity for compulsive checking to develop, perhaps as a compensatory response against hyperactivity. In contrast to the previous study where NAc lesion-saline rats were subjected to only one or two tests (Dvorkin *et al.*, 2010), in the present study, they received 10 trials and still did not develop compulsive checking despite continued hyperactivity. Clearly, the pathogenesis of compulsive checking must involve more than hyperactivity.

#### **Modulation of the focus subcomponent by nucleus accumbens core**

Evidence for the importance of the NAc in activating pathogenesis is limited to two criteria measures only: frequency of checking and recurrence time of checking, each from a different component of compulsive checking. The former criterion measure belongs to the set that defines the vigor component of compulsive checking whereas the latter criterion measure is from the set that defines the focus component of compulsive checking. These effects of NAc lesion are reminiscent of evidence for ‘cross-talk’ between the two measures reported in a study using the serotonin 1A agonist, 8-OHDPAT (Tucci *et al.*, 2014a). It was suggested there that this ‘cross-talk’ may indicate a further split of vigor and focus into subcomponents and that the subcomponents indexed by frequency of checking and recurrence time of checking may reflect interaction between the focus and vigor modules (Tucci *et al.*, 2014a). The design of the present study enables an extension of this interpretation and the suggestion that in addition to its control over vigor, the NAc may modulate the subcomponent of the focus module indexed by recurrence time of checking.

Two lines of evidence suggest such NAc modulation of a subcomponent of focus. One is the observation that in saline-treated rats, the NAc lesion affected one of the two measures defining the focus component – it reduced recurrence time of checking, but did not alter stops before returning to check. This finding was also obtained in a recent acute experiment (Tucci *et al.*, 2014a), but the present study discounts the possibility of a nonspecific effect of surgery because the observed change in recurrence time of checking persisted throughout the course of 5 weeks of testing. Accordingly, it must be considered that the lesion results implicate NAc modulation of recurrence time of checking. The other line of evidence shows that after the NAc lesion, several injections of quinpirole were required to induce enhanced focus (and in particular in the subcomponent of focus indexed by recurrence time of checking), in contrast to merely one or two quinpirole injections for sham controls (Fig. 1c and d). This observation suggests that the action of quinpirole on the NAc normally contributes to enhanced focus and in particular to recurrence time of checking, supporting NAc modulation of a subcomponent of focus. The neuroanatomical substrate for NAc influence over this subcomponent of focus may lie in the connections between the NAc and the OFC, given the proposal that the OFC controls focus and the NAc mediates vigor and satiety (Dvorkin *et al.*, 2010).

In all, the present study suggests that the role of NAc in compulsive checking extends beyond vigor and satiety and includes modulation of a subcomponent of focus. As noted before (Dvorkin *et al.*, 2010), the three identified components of compulsive checking are readily understood within a motivational framework in which compulsive

checking is the exaggerated motor output of a security motivation system. We elaborate on this framework below and propose that the role of the NAc in compulsive checking is to serve as a neural hub coordinating the orderly activity of various neural modules comprising the security motivation system.

### **Nucleus accumbens core and motivational framework of obsessive-compulsive disorder**

A large literature implicates the NAc in motivational processes (Mogenson *et al.*, 1980; Wise, 2005; Salamone *et al.*, 2009; Bromberg-Martin *et al.*, 2010; Cools *et al.*, 2011; Figeo *et al.*, 2011; Da Cunha *et al.*, 2012) and as a site of DBS therapy for OCD (Denys *et al.*, 2010a; Greenberg *et al.*, 2010; Shah *et al.*, 2010; Hamani and Temel, 2012). One might expect, therefore, that a lesion of the NAc would prevent the induction of compulsive checking, but the present study showed that it does not. At first glance, this result appears inconsistent with a motivational framework for quinpirole-induced compulsive checking and hence OCD. However, this is not the case. What the present results show is that an intact NAc is not necessary to develop compulsive checking, suggesting that the neural circuit sufficient for OCD behavior includes brain connections outside the NAc. This conclusion does not contradict the importance of normal NAc function for motivation or that some dysfunction in NAc activity may contribute toward the pathogenesis of OCD or even that DBS at the NAc site may have a normalizing effect on OCD symptoms. Indeed, the findings of the present study suggest that the NAc is a key neural site for the normal function of a special motivation – security motivation – and consequently also for the pathology of OCD, as considered below.

As detailed elsewhere, the security motivation system is a dedicated neural network in the brain that evolved to manage the adaptive challenges of potential threats (Szechtman *et al.*, 2004, 2014; Woody and Szechtman, 2005, 2011, 2013; Szechtman and Woody, 2006). It was theorized that OCD symptoms reflect a breakdown in the mechanism that normally terminates security motivation system activity (Szechtman *et al.*, 2004; Woody and Szechtman, 2005), a prediction supported by recent empirical evidence (Hinds *et al.*, 2012). Of particular relevance to the present study is the claim that species-typical behaviors shown by animals for assessing various domains of potential harm (e.g. Blanchard and Blanchard, 1988; Curio, 1993; Wingfield *et al.*, 1998; Lima and Bednekoff, 1999) are the output of an activated security motivation and include probing/exploring the environment when the stimulus of potential threat is deviation from safety such as unfamiliar surroundings (for a detailed description of the security motivation system, including its proposed neuroanatomy and physiology, see: Woody and Szechtman, 2011). Hence, it follows that the description of the behavior of animals confronted

with an environment outside the safety of their territory (a large open field) is an account of the output of an activated security motivation. We use the elegant work of others (Eilam *et al.*, 1989; Golani *et al.*, 1993; Golani, 2012; Weiss *et al.*, 2014) who analyzed the behavior of rats placed in a large open field as our description below of the typical response of an activated security motivation.

That the NAc is required for the proper function of security motivation is shown by the striking transformation in open-field behavior of saline-treated rats with a NAc lesion. These animals did not behave as normal rats do when security motivation is activated upon exposure to a novel environment. Normally, the security motivation response to the potential dangers of a novel space is systematic probing and checking of the environment, which begins with the rat choosing one spot as its home base and using this locale as a focal point to organize successively wider excursions to probe the unfamiliar surroundings; these probes consist of round trips from the home base with several stops along the excursion route before returning to the home base; typically, the rat stays at the home base a fair amount of time before embarking on another round trip, usually by retracing recently traveled paths; and finally, the rat settles at the home base once probing of potential danger is satisfied and the environment is familiar (Eilam *et al.*, 1989; Golani *et al.*, 1993; Golani, 2012; Weiss *et al.*, 2014). The profile of saline-treated NAc lesion rats was different. Lesion rats spent less time in the home base (evidenced by their shorter ‘duration of visit to key locale’; Fig. 1b); less time outside the home base (evidenced by shorter ‘return time to key locale’; Fig. 1c); and embarked on more round trips (evidenced by a higher ‘number of returns to key locale’; Fig. 1a) although without any change in the number of stops along the excursion route (no lesion-induced change in ‘number of stops between returns to key locale’; Fig. 1d). Moreover, the saline-treated NAc-lesion rats were active continually without settling down in the home base to rest for a normal period of time (evidenced by reduced ‘time to next checking bout’ and elevated distance traveled; Fig. 1d and Fig. 3a). Such lesion-induced changes show that for security motivation to operate properly, the NAc must be intact to perform some critical function.

What functional role does the NAc contribute toward the normal operation of security motivation? The present study is consistent with suggestions that the NAc normally serves as a hub for coordinating the orderly activity of neural modules mediating the various components of motivational systems (Mogenson *et al.*, 1980), including, we suggest here, security motivation. As noted, the security motivation system comprises at least three functional components: a ‘vigor’ component that controls the intensity with which motor acts of checking and probing are performed; a ‘focus’ component that directs probing to relevant areas of the environment; and a

‘satiety’ component that controls termination of security-related activity and rest after deactivation of security motivation (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a). Previous findings suggested that the NAc exerts inhibitory control over motivational vigor and satiety components (Dvorkin *et al.*, 2010; Tucci *et al.*, 2014a), a suggestion based on a NAc lesion-altered probing profile as replicated here (shorter acts, many repetitions, little rest). The present study suggests that the NAc also controls a subcomponent of the focus module.

Thus, the present findings from saline-treated and quinpirole-treated rats show that the NAc is involved in the proper operation of security motivation as well as contributing toward the developmental process by which repeated injections of quinpirole exaggerate the function of all three constitutive components of compulsive checking. The finding that the NAc can be involved in all three components of security motivation and compulsive checking suggests that the NAc is well positioned as a neural hub for coordinating the proper initiation, maintenance, and termination of activity in the various components of the security motivation system. Considering that the neural circuitry of the security motivation system encompasses a network of corticobasal ganglia loops with many brain regions (Woody and Szechtman, 2011; Szechtman *et al.*, 2014), we propose that one functional role of the NAc is to recruit and engage relevant brain areas outside the NAc as needed by the security motivation system. Consistent with this hypothesis are experiments showing that the NAc is an effective site for electrical stimulation to engage and arrest oscillatory activity across the neural network of corticobasal ganglia loops (McCracken and Grace, 2007; Brittain *et al.*, 2014), indicating that focal NAc activation exerts widespread network effects (Rauch *et al.*, 2006; McIntyre and Hahn, 2010; Figeo *et al.*, 2013). Because of this property, our hypothesis can account for how the nucleus accumbens can be a site for OCD therapy (as with DBS) and yet contribute toward OCD pathology, even if the pathogenesis of OCD lies elsewhere in the brain.

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## Conflicts of interest

There are no conflicts of interest.

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