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expression of quinpirole-induced compulsive checking was full-blown. Analysis of Golgi stained neurons showed changes in spine density in Cg3 and Par1 and increased branching of apical dendrites in Cg3. It is suggested that compulsive checking could be coupled to drug-induced increases in Cg3 dendritic branching and that changes in spine density may reflect a compensatory adjustment in dopamine-innervated regions. On the basis of the animal model findings, it is concluded that the presence of OCD checking compulsions is not dependent on pituitary axis hormones.

Effects of hypophysectomy on compulsive checking and cortical dendrites in an animal model of obsessive-compulsive disorder

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Abstract

Hormones may modulate the symptoms of obsessive-compulsive disorder (OCD) but the evidence is equivocal and not consistent across studies, with findings of hormone-associated increases and decreases of symptoms. To assess whether a strong endocrine influence on OCD exists, the effects of hypophysectomy were examined in an animal model of OCD. The model involves repeated injections of the dopamine D2/D3 receptor agonist, quinpirole, to induce locomotor sensitization and compulsive checking behavior. Intact and hypophysectomized rats were administered quinpirole (0.5 mg/kg x6, twice weekly) or saline and compulsive checking in a large open field was measured according to a standard protocol. Results showed that in hypophysectomized animals the development of locomotor sensitization was attenuated but the expression of quinpirole-induced compulsive checking was full-blown. Analysis of Golgi stained neurons showed changes in spine density in Cg3 and Par1 and increased branching of apical dendrites in Cg3. It is suggested that compulsive checking could be coupled to drug-induced increases in Cg3 dendritic branching and that changes in spine density may reflect a compensatory adjustment in dopamine-innervated regions. On the basis of the animal model findings, it is concluded that the presence of OCD checking compulsions is not dependent on pituitary axis hormones.

Key words: Rat; Hypophysectomy; Sensitization; Locomotion; OCD; Dendritic morphology; Structural plasticity; Psychostimulant drugs

Introduction

Chronic treatment with the D2/D3 dopamine agonist quinpirole induces in rats marked locomotor sensitization, as evidenced by a 4-8 fold higher amount in distance traveled at injection 10 compared to acute quinpirole injection (Willner *et al.,* 1992; Mattingly *et al.,* 1993; Szechtman *et al.,* 1994b; Brus *et al.,* 2003). In a large open field (1.6 m x 1.6 m), quinpirole induces together with locomotor sensitization a transformation in the spatiotemporal structure of the rats' activity. The profile of this change has led to the suggestion that the quinpirole-sensitized rat is engaged in compulsive checking behavior, and to the proposal that behavior under quinpirole constitutes an animal model of obsessive-compulsive disorder (OCD) (Szechtman *et al.,* 1998; Man *et al.,* 2004; Eilam & Szechtman, 2005; Joel, 2006; Korff & Harvey, 2006; Westenberg *et al.,* 2007). This model has revealed the importance of cholinergic systems (Tizabi *et al.,* 1999; Brus *et al.,* 2003; Brown *et al.,* 2004) in the display of quinpirole-induced compulsive checking (Tizabi *et al.,* 2002), a finding that supported a human study to investigate the effects of a nicotine patch on OCD symptoms (Salin-Pascual & Basanez-Villa, 2003; Lundberg *et al.,* 2004). The model has been similarly useful in pointing to an inhibitory factor in governing the duration of checking (Dvorkin *et al.,* 2006b) and to a role of kappa opioid receptors in accelerating the development of compulsive checking behavior (Perreault *et al.,* 2007). It has been used also for evaluation of deep brain stimulation (DBS) of the subthalamic nucleus in ameliorating compulsive checking (Winter *et al.,* 2008). In the present study, we use the quinpirole model of OCD to ask two questions: 1) whether compulsive checking is dependent on pituitary hormones, and, 2) whether the expression of compulsive checking and sensitization to quinpirole is related to a specific change in synaptic plasticity. While the two questions are theoretically unrelated they are presented together in this report

because they were addressed in a single experiment and because their juxtoposition contributes towards illuminating the obtained findings. Below we address the rationale for each question in turn.

As reviewed by McDougle et al (1999), a number of clinical studies assessed the levels of posterior and anterior pituitary hormones in relation to severity of OCD symptoms. Unfortunately, the obtained findings were inconclusive: the reported relationship to OCD for vasopressin, oxytocin and ACTH were generally contradictory across studies. Other studies pointed to fluctuations in OCD symptoms across the menstrual cycle and pregnancy (Williams & Koran, 1997; Labad *et al.,* 2005; Vulink *et al.,* 2006), suggesting a possible contribution of pituitary-gonadal hormones in OCD. However, again, results were bidirectional, showing both an exacerbation and an attenuation in symptoms in relation to the reproductive cycle. Finally, there is some evidence in OCD patients of a differential response for TSH (Aizenberg *et al.,* 1991) and prolactin (Fineberg *et al.,* 1997; Monteleone *et al.,* 1997) to challenge with TRH and fenfluramine, respectively. However, such challenge tests do not indicate whether differences between patients and controls pertain to hormonal effects on OCD symptomatology or to neuroendocrine control over hormone release *per se*. Overall, most of the reviewed clinical studies precluded conclusive evidence for a cause-effect relationship between hormones and OCD symptoms because their experimental design provided correlational data only.

Considering that the putative clinical effects of hormones on OCD symptoms were not very robust across studies, we reasoned that a strong endocrine effect could be demonstrated by the total elimination of hormones, as provided by hypophysectomy. Therefore, the present study examined the effects of hypophysectomy on the development of compulsive checking induced by repeated injections of quinpirole. Because quinpirole-induced compulsive checking is

dependent on the attainment of locomotor sensitization (Eilam & Szechtman, 2005), we employed groups of control and hypophysectomized rats already sensitized to quinpirole (Culver & Szechtman, 2004). These rats had their sensitization treatment in activity chambers, where compulsive checking cannot be measured due to the small size of the apparatus. Accordingly, in the present study, animals received additional tests in a large open field to measure compulsive checking (Dvorkin *et al.,* 2006b).

The present study had an additional purpose related to the question whether there exists a reorganization of synaptic connections produced by quinpirole. In a recent series of studies reviewed in Robinson and Kolb (2004), a strong correlation was observed between sensitization produced by chronic treatment with psychostimulant drugs, such as amphetamine and cocaine, and several types of changes in dendritic morphology. These correlations suggest that at least some of the morphological changes may underlie sensitization but which ones specifically remains to be established. Because quinpirole also induces sensitization, it may be expected that chronic treatment with this drug should produce changes in dendritic morphology and the present study sought to verify this prediction. Moreover, because quinpirole has a different mode of action than amphetamine and cocaine (quinpirole is a direct dopamine receptor agonist while amphetamine and cocaine are indirect agonists that elevate synaptic dopamine) a comparison between the effects of quinpirole on dendrite morphology with those of the indirect agonists should prove informative in separating morphological changes specific to particular psychostimulant drugs from those that are necessary for sensitization across drugs. Furthermore, the opportunity to evaluate in the present study dendritic changes in intact and hypophysectomized rats should provide new data on the effects of hypophysectomy on structural plasticity as such information is very sparse in the literature. In addition, by virtue of having a

greater number of treatment groups, the comparison between intact and hypophysectomized rats should help to reveal which morphological changes are specific to quinpirole effects on sensitization and compulsive behavior and which morphological effects are not necessarily linked to quinpirole effects on behavior.

The brain regions that have been typically examined in studies on the effects of psychostimulant drugs on dendrite morphology (Robinson & Kolb, 2004) include target areas of dopamine innervation such as the medial prefrontal cortex and the nucleus accumbens; the parietal cortex is generally also measured and serves as a control region with minimal dopamine innervation. Based on this prior literature we examined in the present report the medial prefrontal cortex (Cg3) and the parietal cortex (Par1).

Methods

Subjects

Twelve hypophysectomized male Long-Evans rats (Charles-River, Canada) and twelve control (surgically intact) male Long-Evans rats (Charles-River, Canada) were used. These rats were a subset of animals used in a previous study examining the effects of clorgyline on sensitization to quinpirole (Culver & Szechtman, 2004). Specifically, from the non-clorgyline treated (intact and hypophysectomized) 4 control groups used by Culver & Szechtman (2004), a random sample of 6 rats was selected from each group to continue with its treatment regimen in the current experiment. As noted in the Procedure section, there was no interruption between the previous and the current experiments, with the first open field test being conducted 7 days after the last test in activity chambers (analysis of the current experiment awaited the completion of computer software for measuring compulsive checking from video records; Dvorkin *et al.,* 2006b). At the time of their first test in the open field, hypophysectomized and intact rats

weighed 140±3 gm and 440±11 gm, respectively; their corresponding weights at the last test were 148±4 gm and 520±14 gm (mean±SEM). Rats were individually housed in a temperature controlled colony room (22°C) under a 12 h light-dark cycle, with free access to food and water. The drinking water of hypophysectomized rats consisted of a 5% sucrose solution, as prescribed for hypophysectomized rats (http://www.criver.com/research_models_and_services/surgical_services/faq_rodent_surgery.html). Rats were allowed to acclimatize to the colony room for 1 week following arrival and were handled 2 minutes daily for 7 days before the start of the experiment. All treatment and testing was conducted during the light-on hours. Animals were housed and tested in compliance with the guidelines described in the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

Surgery

All hypophysectomies were performed using the parapharyngeal approach by Charles-River, Canada (order code: HYPOX), approximately 1 week before shipment of the rats to McMaster University. In rats, a complete hypophysectomy is indexed entirely by an absolute absence of body weight gain or a gain that does not exceed 10 g (Hahn *et al.,* 1965; Bellinger *et al.,* 1979), and thus measurement of pituitary hormones is not necessary to confirm the status of hypophysectomy. Consequently, in the present study, hypophysectomy was considered successful if rats showed no, or minimal (less than 10 g) weight gain. All rats in the current experiment satisfied that criterion. Control rats did not undergo sham surgery because the supplier did not offer such an option and because sham surgery for non-hypophysectomy procedures in other studies did not produce any enhancing or suppressive effects on quinpirole sensitization (Culver & Szechtman, 2004).

Drugs

Quinpirole hydrochloride (RBI, Natick, MA) was dissolved in saline and injected subcutaneously at a dose of 0.5 mg/kg, twice weekly (every 3-4 days). This quinpirole dose and injection regimen were selected because the 0.5 mg/kg dose is representative of the behavioral effects induced by quinpirole doses ranging from 0.25 to 2.5 mg/kg, and because the locomotor effects of chronic treatment reach a plateau after 8-10 drug injections administered 2 to 8 days apart (Eilam & Szechtman, 1989; Szechtman *et al.,* 1994a; Szechtman *et al.,* 1994b).

Apparatus

Animals were tested in a large open field consisting of a solid surface top table (160 x 160 and 60 cm high). The table top was constructed of material used in making kitchen counter-tops - it was smooth, non-porous, composed of unsaturated polyester and acrylic resin blends (Acryflek Industries; http://www.acryflek.ca/product/index.shtml), and had a custom blue color to facilitate video detection of dark and white objects. Four small Plexiglas/glass boxes (approximately 8 x 8 x 7.5 cm) were present at the same fixed location of the open field throughout the study: two at corners and two at places near the center of the open field. The open field platform was virtually subdivided into 25 rectangular places (locales) used to define the location of the animal in the field. The open field and objects were wiped clean after each rat with a diluted solution of an ammonium cleaner (Windex). Behavior was videotaped continuously by a camera (Ikegami ICD-47) affixed to the ceiling, providing a stationary top view of the entire open field and the rat in it. Videotapes were converted to MPEG files (Canopus MPEGPro EMR realtime MPEG-1 MPEG-2 encoder) and these digitized videos were used to automatically track the trajectories of locomotion in the open field using EthoVision 3.1 (Noldus Information Technology bv, Netherlands) system (Noldus *et al.,* 2001; Spink *et al.,*

2001). The spatial sensitivity of the tracking system was 8 mm x 8 mm per pixel, with a temporal resolution of 30 frames per second.

Procedure

Prior to the current experiment, rats were injected twice weekly with either quinpirole (QNP), or saline (SAL) for a total of 8 injections, and following each injection their locomotor activity was measured in small activity chambers. Animals received 3 additional tests in activity chambers where they were injected with 0, 0.07 and 0.2 mg/kg of quinpirole in random order (Culver & Szechtman, 2004). A week later, the current experiment begun with animals continuing with their prior treatment regimen of quinpirole (0.5 mg/kg) or saline, except that now they were tested in the open field following each injection, for a total of 6 injections. Four groups of rats (N=6/group) were used: NS, intact + saline; NQ, intact + quinpirole; HS, hypophysectomized + saline; HQ, hypophysectomized + quinpirole. On the day of testing, animals were weighed, transported in their home cage to an adjoining non-colony testing room, and administered the appropriate injection. Immediately afterwards, rats were placed into the open field for 55 minutes and their behavior was videotaped for offline analysis.

Behavioral analysis

Locomotion in the open field

Two aspects of locomotor activity in the open field were quantified: distance traveled and spatial distribution of locomotor trajectories. The input data for these assessments, as well as for those of checking behavior (see below), were the time series of *x,y* coordinates of the rat's center of gravity in the open field (or more precisely, the centroid of the horizontal two dimensional image of the rat body), as extracted by EthoVision 3.1 software from the digitized video recordings. The details by which these track files of *x,y* coordinates are processed to compute

the requisite measures were described previously (Dvorkin *et al.,* 2006a) and were followed here. The following three dependent variables were evaluated: *total distance traveled; path stereotypy ratio* (a measure of the relative frequency of repetitions of travel along the same paths); and, *area of 2 standard deviational ellipse (2SDE*; a measure of the extent of the space covered by the trajectories of locomotion).

Checking behavior

Using a virtual implementation of the coordinate system of 25 open field locales (places) (Szechtman *et al.,* 1998), the EthoVision trackfiles were processed as described previously (Dvorkin *et al.,* 2006b), and the frequency of visits and the duration of stops in each locale were computed (the terms "visit" and "stop" are equivalent and are used interchangeably). The obtained values were used to identify the locale with the highest cumulative frequency of visits as well as the place with the maximal cumulative duration of stops. In almost all instances for quinpirole-treated rats (Szechtman *et al.,* 1998), and generally for saline-treated rats (Eilam & Golani, 1989), the locale with most visits is also the locale with longest duration of stops. Checking behavior was defined with reference to the most visited locale (Szechtman *et al.,* 1998), here called *key place* or *key locale*. If several locales had an equal number of visits then the locale with the higher cumulative duration of stops was used as the key locale. The following measurements of checking behavior were computed from the processed EthoVision trackfiles (a visit to key place is also referred to as a "check" or "checking"): absolute and relative frequency of checking the key place, absolute and relative checking recurrence time, number of locales visited before returning to the key place, and mean duration of visits to key place. These dependent variables were defined during previous studies of checking behavior (Szechtman *et al.,* 1998; Szechtman *et al.,* 2001; Tizabi *et al.,* 2002; Dvorkin *et al.,* 2006b). As

reviewed elsewhere (Szechtman & Eilam, 2005; Eilam & Szechtman, 2005), these measures are said to reflect two characteristics of compulsive checking—a preoccupation with the performance of the behavior and a reluctance to leave the place/object on which the behavior is focused. *Absolute frequency of checking* is the total number of visits to the key locale while the *relative frequency of checking* is the rate of checking that is computed by dividing the observed number of visits to the key locale by the expected number of stops per visited locales (the relative or rate measurement controls statistically for group differences in the overall amount of activity). *Absolute checking recurrence time* is the mean duration of return times to the key place, where return time is the interval from departure to the next arrival to the locale; *relative checking recurrence time* is the mean return time to the key place normalized to the mean of return times to every place that was visited more than once. *Number of locales visited before returning to the key place* is the mean number of stopping places in between returns to the key locale. Finally, *mean duration of visits to key place* (that is, *mean length of checks*), is the total duration of stay at the key locale divided by the frequency of visits there; this measures is also an indirect index of ritual-like behavior as very short duration of stay in the key locale is associated with appearance of motor rituals in quinpirole rats (Szechtman *et al.,* 1998; Ben Pazi *et al.,* 2001). Compulsive checking behavior is identified by the presence of a significant difference between quinpirole- and saline-treated rats on all the above measures (Szechtman *et al.,* 1998).

Analysis of dendrites and spines

Within a week of completion of testing, rats were anesthetized with isoflurane and transcardially perfused with 0.9% saline. The brains were removed and placed in vials containing Golgi-Cox solution (Kolb & McClimans, 1986), and after 14 days transferred to vials containing 30% sucrose. After several days in sucrose, the brains were cut into 200 μm coronal

sections and stained according to procedures described elsewhere (Gibb & Kolb, 1998). Pyramidal cells in layer III of the parietal cortex [Zilles' area Par 1; (Zilles, 1985)] and in layer V of the medial prefrontal cortex (Zilles' area Cg3) were traced using a camera lucida drawing tube, magnified at 250x, that was attached to the microscope. To be included in the data analysis, the dendritic trees of pyramidal cells had to fulfill the following criteria: (a) the cell had to be well impregnated and not obscured with blood vessels, astrocytes, or heavy clusters of dendrites from other cells and (b) the apical and basilar arborizations had to appear to be largely intact and visible in the plane of section. The cells were analyzed by drawing the cells using camera lucida. To quantify the extent of dendritic arborization, the number of dendritic branches was determined according to the procedure of Coleman and Riesen (1968), and the length of dendrites was estimated by using the procedure of Sholl (1967) and multiplying the number of crossings by 20 μm. Spine density was measured from one apical dendritic branch in the terminal tuft, and from the secondary branch proximal to the cell body for one basilar branch, following the procedure of (Woolley *et al.,* 1990). Spine density measures were made from a segment greater than 10 μm in length. The dendrite was traced at 1000x using a camera lucida drawing tube, and the exact length of the dendritic segment was calculated. Spine density was expressed as the number of spines per 10 μm. Because we did not attempt to correct for spines hidden beneath or above the dendritic segment, the spine density values likely underestimated the actual density of the dendritic spines. Each measure of dendritic arborization and of spine density was obtained independently from the left and the right hemispheres. Five cells in each region in each hemisphere of each rat were drawn and the computed mean represented a score for a given hemispheric region.

Statistics

To analyze the effects of hypophysectomy on the behavioral response to repeated injections of quinpirole, a three-way ANOVA was performed, with two between-group factors: Hypophysectomy (intact *versus* hypophysectomized) and Chronic Drug Treatment (saline *versus* quinpirole) and a repeated measures factor, Injection Number (injection 1 to injection 6). For the dendrite morphology data, the results of Hypophysectomy by Chronic Drug Treatment ANOVAs are reported as preliminary statistical analyses showed that the factor of left *versus* right hemisphere did not yield any statistically significant effects. Separate analyses were employed for each dependent variable. When a significant interaction was followed by post-hoc tests, simple effects were evaluated by comparing the relevant marginal means and 95% confidence intervals and non-overlapping means considered as statistically significant. The chosen level of significance was $P < 0.05$. Calculations were performed using SPSS 15 for Windows.

Results

Locomotor sensitization in the open field

Amount of locomotion

As shown in Fig. 1a, in the open field, both the hypophysectomized and the intact rats treated with quinpirole locomoted substantially more than the saline treated groups, from the very first injection and onwards (for the main effect of Drug, $F(1,20) = 51.2$, $P < 0.001$). However, the hypophysectomized rats did not increase the amount of locomotion across quinpirole injections in contrast to the intact group that did show significantly more locomotion at injections 5 and 6 compared to the first injection of quinpirole (for Injection x Hypophysectomy x Drug, $F(5, 100) = 4.6$, $P = 0.001$). This may suggest that the intact quinpirole-treated rats exhibited locomotor sensitization, but hypophysectomized rats did not. In

fact, both groups were well sensitized at the start of open field testing, as indicated by the following two observations:

(1) The response to quinpirole upon the first open field injection was 4-6 fold higher than the response to saline (Fig. 1a). This amount of locomotion is comparable to a fully sensitized locomotor response and much greater than a typical response to acute quinpirole injection (Szechtman *et al.,* 1994b; Dvorkin *et al.,* 2006b);

(2) In activity chambers that preceded testing in the open field (see Fig. 1b), both hypophysectomized and intact groups showed a 6-8 fold rise in locomotor response to quinpirole at injection 8 compared to their acute injection, consistent with the typical profile indicative of locomotor sensitization (Szechtman *et al.,* 1994a).

Thus, while both groups entered the open field at equivalent levels of locomotor sensitization, the intact animals sensitized even more across quinpirole injections but the hypophysectomized rats remained at their starting level of sensitized responding. The saline-treated rats—intact and hypophysectomized—showed no significant change across injections and were not different from each other (Fig. 1a).

Spatial distribution of locomotion

A similar distinction between intact and hypophysectomized rats was evident also in the spatial distribution of their locomotor activity, shown in Fig. 2. Specifically, both intact and hypophysectomized rats treated with quinpirole exhibited greater path stereotypy than the groups treated with saline (for the main effect of Drug, $F(1,19) = 89.9$, $P < 0.001$). Inspection of the path stereotypy graph (Fig. 2, lower panel) suggests that only in intact rats there was a further augmentation of path stereotypy across quinpirole injections. This observation was supported by comparisons of simple effects, indicating a significant ($P < 0.05$) difference between injection 1

and injections 4-6, although the strength of this statistical evidence may be questioned due to the lack of a significant interaction for Injection by Hypophysectomy by Drug ($F(5, 95) = 0.87$, $P = 0.5$). Nevertheless, an augmentation of path stereotypy across quinpirole injections can be also discerned from an inspection of the upper panel of Fig. 2, which shows the routes of locomotion after each injection for a representative rat from each group. Plots with darker areas indicate greater travel along the same trajectory. As is evident, the quinpirole-treated rats traveled repeatedly along the same trajectories in contrast to the saline animals that showed only few such paths. Furthermore, Fig. 2 (upper panel) shows that in the intact quinpirole rat (NQ), but not in the hypophysectomized animal (HQ), the plots for routes of frequent travel are more dark during later injections of the drug, suggesting more repetitions of travel along the same path, a suggestion that is consistent with the quantitative profile of the path stereotypy index in Fig. 2, lower panel.

Inspection of the routes (Fig. 2, upper panel) suggests the presence of yet another difference in the spatial distribution of locomotor paths. As shown, the routes of frequent travel in quinpirole rats were concentrated within a relatively small area while in the saline rats the routes were distributed relatively evenly over the entire open field, suggesting that quinpirole rats confined their locomotion to a more constricted area of the open field. This impression is supported by analysis of 2 standard deviational ellipse (2SDE), a measure that indexes the spatial extent of the area over which trajectories are spread. As shown in Fig. 2 (lower panel), 2SDE was significantly smaller in quinpirole- than saline-treated rats (for Drug, ($F(1,20) = 29.3$, $P < 0.001$), consistent with spatial constriction in quinpirole rats. Furthermore, there were no main effects of hypophysectomy or injection (or interaction effects) suggesting that spatial spread was not affected by the absence of pituitary hormones nor did it sensitize across

quinpirole injections. The lack of change in 2SDE across injections is similar to previous findings (Dvorkin *et al.,* 2006a; Dvorkin *et al.,* 2006b). The fact that the values for 2SDE obtained in the present study are similar to those previously reported (Dvorkin *et al.,* 2006a; Dvorkin *et al.,* 2006b) provides additional assurance for the reliability of the present findings.

Time course of locomotion

Fig. 3 provides additional evidence that both the intact and the hypophysectomized quinpirole-treated groups were already well sensitized upon their first introduction into the open field. Normally, there is a biphasic response to quinpirole—locomotor inhibition followed by excitation (Eilam & Szechtman, 1989; Eilam *et al.,* 1989). As sensitization develops with repeated injections of quinpirole, the excitatory phase increases and its onset advances gradually to within 5 minutes after drug injection, thereby shortening and supplanting the inhibitory phase (Szechtman *et al.,* 1994b; Perreault *et al.,* 2006). As can be seen in the lower panel, both the NQ and HQ groups showed no inhibition of locomotor activity between 5 and 20 min after quinpirole injection, consistent with a sensitized response to quinpirole.

The time-course data in Fig. 3 provides further information bearing on the finding presented in Fig 1a that for total distance, intact rats, but not the hypophysectomized group, showed added sensitization across repeated quinpirole injections. Specifically, the time course graphs suggest that the Fig 1a data are somewhat more complex and that in fact both intact and hypophysectomized rats had similar increases in the amount of locomotion during the first 20 min, and that only later on, in the last part of the session, did a difference between the intact and hypophysectomized quinpirole rats emerge. This profile can be seen clearly in Fig. 3 (inset) that shows the amount of locomotion from 5 to 20 min and from 20 to 55 min after each drug injection. As shown, during the first time period, intact and hypophysectomized animals showed

a similar and significant regression slope for locomotion across injections, indicating equivalent sensitization across quinpirole injections. However, during the second time period the regression slope was significant for intact animals only, indicating yet further locomotor sensitization in intact but not in hypophysectomized rats (Fig. 3, inset).

Thus, results indicate that rats sensitized to quinpirole in small activity chambers retain their sensitization upon introduction into the open field. With repeated injections of quinpirole in the open field, intact animals show further sensitization which spans the entire session, while the increases in locomotion for hypophysectomized animals are confined to the first 20 min after drug injection.

Checking behavior

According to the criteria defining compulsive checking behavior (see Methods), intact and hypophysectomized groups exhibited compulsive checking from the first quinpirole injection in the open field (Fig. 4; main effect of Drug for each measure, $P < 0.05$; Table 1) but it is not obvious whether checking behavior showed further augmentation across the six quinpirole injections. The statistical finding of a significant Drug by Injection interaction for each measure of checking behavior except length of check (Table 1), may suggest further sensitization. However, inspection of Fig. 4 contradicts such an interpretation because measures of checking showed relatively little change across quinpirole injections. To resolve this apparent contradiction and identify the source for the Drug by Injection interaction, the data were re-analyzed with injection 5 omitted because inspection of Fig. 4 suggests unusual performance on this particular test for one or more groups. Upon re-analysis, a significant Drug by Injection interaction was now present for only two measures, frequency of checking ($F(4,72) = 2.99$, $P = 0.024$; Fig. 4a) and stops before returning to key locale ($F(4,72) = 5.9$, $P < 0.001$; Fig 4e).

Evaluation of the relevant means suggested that in the case of frequency of checking (Fig 4a), the interaction effect likely reflected a statistically insignificant change of means across injections, increasing in the quinpirole-treated rats and declining in rats treated with saline. For stops before returning to key locale (Fig 4e), the Drug by Injection interaction resulted from a significant decline across injections in saline-treated animals (5.4±0.3 stops at injection 1 *vs* 3.4±0.2 stops at injection 6, $p < 0.05$), but no significant change across injections in quinpirole-treated rats. Thus, overall, it appears that compulsive checking behavior was present from the first test in the open field but showed no further augmentation with repeated testing.

In terms of the effect of hypophysectomy itself, the consequence of hypophysectomy on compulsive checking was selective as only one measure, stops before returning to key locale (Fig. 4e), showed a statistically significant main effect of Hypophysectomy ($F(1,18) = 4.4$, $P = 0.051$) and interaction effect (for Drug by Hypophysectomy by Injection, $F(5,90) = 4.8$, $P = 0.001$). One interpretation of such a result is that hypophysectomized rats were more attracted to their place of interest because they made fewer visits elsewhere before returning to the key locale (Fig 4e). This may suggest increased compulsive checking in hypophysectomized rats, a notion consistent with the trend for a higher relative frequency of checking of the key locale in the hypophectomized quinpirole group (for Hypophysectomy by Drug, $F(1,18) = 2.7$, $P = 0.115$; Fig 4b). However, because a statistically significant change was found in only one measure of checking behavior, the data provide only weak evidence for greater compulsiveness in hypophysectomized rats.

Morphology of dendrites

As shown in Fig. 5, hypophysectomy had a marked stimulatory effect on dendrite morphology, increasing the number of spines on apical and basilar dendrites of neurons located

in both brain regions examined, the medial prefrontal cortex (Cg3) and the parietal cortex (Par1). Moreover, hypophysectomy increased arborization of basilar dendrites in the parietal cortex, as measured by the number of branches and the length of dendrites.

The effects of quinpirole were more complicated, in that quinpirole produced region-dependent increases or decreases in dendrite morphology, and in that the direction of some changes was influenced by hypophysectomy. In particular, quinpirole treatment had opposite effects on spines in Cg3 and Par1, decreasing their number in Cg3 and increasing in Par1. Moreover, for apical dendrites in the Cg3 region, the effects of quinpirole were to increase the number of branches. However, for basilar dendrites in both Cg3 and Par1, the effects of quinpirole on dendritic arborization were dependent on the presence of the pituitary gland, as evidenced from the four significant Drug by Hypophysectomy interactions depicted in Fig. 5. These interactions resulted from the fact that in hypophysectomized rats, quinpirole treatment had a stimulatory effect on branching and dendritic length in Cg3 and on branching and spine density in Par1, whereas in intact rats, quinpirole had an inhibitory or no effect at all on the same measures.

Discussion

Compulsive checking and hormones

The present study provides quite clear evidence that in the absence of pituitary axis hormones, as obtained through hypophysectomy, there is nevertheless robust demonstration of compulsive checking in a rat model of OCD. Given that this model involves repeated injections of a D2/D3 dopamine agonist and the development of sensitization, the quinpirole preparation is in essence a dopamine sensitization model focused on compulsive checking in OCD. Consequently, findings from the quinpirole model suggest that for subtypes of OCD involving

dopaminergic activity (Denys *et al.,* 2004; Denys *et al.,* 2006), the presence of OCD symptoms is not dependent on pituitary axis hormones.

At first glance, the above conclusion from the animal model may appear at variance with human data, which albeit somewhat inconsistent, does nevertheless point to differences in aspects of OCD symptomatology in relation to stages of the reproductive cycle (Williams & Koran, 1997; Maina *et al.,* 1999; Labad *et al.,* 2005; Vulink *et al.,* 2006) or gender (Bogetto *et al.,* 1999; Lochner *et al.,* 2004). Moreover, there is even a report in mice suggestive of an influence of estrogen on the amount of grooming that the authors posit could be related to compulsive washing in OCD (Hill *et al.,* 2007). But despite the appearance, findings from the quinpirole model do not necessarily present a contradiction and in fact may help to illuminate the clinical results, as discussed below.

One interpretation of the quinpirole model and clinical data is the possibility that the animal and human studies captured a different aspect of OCD, differing in the relationship to hormones. For instance, an important distinction had been made between the *structure* and the *content* of OCD as OCD symptoms appear to share a common form across patients but the concerns or specific content of obsessive thoughts and compulsive rituals vary widely from patient to patient (Reed, 1985). As noted by Saad (2006), studies examining a possible relationship between hormones and OCD focused on content, in that specific OCD concerns (especially those related to contamination) were shown to vary as a function of reproductive status/gender; the author hypothesized an evolutionary basis for sex-dependent links to specific concerns (see also, Feygin *et al.,* 2006). Accordingly, the human clinical data may be said to reveal that the focus of sex-dependent OCD concerns is modulated by hormonal status, in concert with such hormonal influence on normal concerns. In contrast, the results from the

animal model – a model which apriori was designed to capture the spatiotemporal structure of OCD compulsions (Szechtman *et al.,* 1998) – may be said to emphasize that the existence of OCD pathology is possible even in the absence of pituitary axis hormones. Thus, a juxtaposition of the animal model and clinical data highlights the possibility of greater hormonal influence over the content/focus of OCD concerns than over the form of the psychopathology.

Although compulsive behavior can be expressed without pituitary axis hormones, this does preclude the possibility that normal fluctuations in hormone levels could contribute to sculpturing OCD performance, especially in a susceptible subpopulation (Schneider & Popik, 2007). A slight hint of such a possibility is provided by the finding of a significant effect of hypophysectomy on one measure of compulsive checking (Fig. 4e) and a trend for a similar change in another one (Fig. 4b). These changes may be interpreted as reflecting increased compulsiveness because they index a higher than usual focus on the key locale, the putative focus of checking. However, this interpretation must be viewed as highly speculative because the identification of compulsive checking (Szechtman *et al.,* 1998) involves a significant change in all measures depicted in Figure 4, and thus the meaning of a change in only one or two of them is not yet understood.

Hormonal modulation of locomotor sensitization

Hypophysectomy had a relatively strong negative effect across injections on the kinetics of locomotion after an injection of quinpirole. In intact quinpirole-treated rats, locomotion rose continually from injection to injection. In hypophysectomized rats, the profile was similar but only for the first 20 min of quinpirole injections; from 20 min onwards the amount of locomotion remained at the same steady level from injection to injection (Fig. 3). One plausible interpretation for this distinction may be related to the observation that chronic quinpirole

treatment shifts nutrient utilization away from carbohydrates and towards fat mobilization (Coscina *et al.,* 1998) and this source of energy may be needed for sensitized locomotion. Because hypophysectomized animals do not gain weight (Hahn *et al.,* 1965; Bellinger *et al.,* 1979; Culver & Szechtman, 2004), they are limited in the supply of lipids to generate energy. Consequently, hypophysectomized rats may be comprised in their ability to generate sufficient energy to fuel full-blown locomotor sensitization to quinpirole.

It is interesting to note from an inspection of Fig. 1 and Fig. 3 (inset) that on their first exposure to the large open field, the intact and the hypophysectomized rats had similar amounts of locomotion, consistent with their similar performance on the previous 8 injections in the small activity cages. Thus, hypophysectomized rats were equally sensitized upon exposure to the new environment but did not sensitize further to the same extent as controls. The apparent attenuation of further sensitization may be related to the passage of time after hypophysectomy and the associated decline in energy or other resources needed to promote locomotor sensitization. Alternatively, further sensitization may have been attenuated because the new environment presented additional opportunities for locomotor development with higher energy load (e.g., attaining higher running speeds) but hypophysectomized animals were disadvantaged because of their energy supply limitations. Of course, some combination of both is also possible.

Structural plasticity, hypophysectomy and compulsive checking

The strongest effect of hypophysectomy observed in the present study was to stimulate dendritic growth, increasing the number of dendritic spines in the medial prefrontal (Cg3) and parietal (Par1) cortices, as well as increasing branching and length in basilar dendrites of neurons in Par1. The extensive effects of hypophysectomy on dendritic morphology contrast with the more subtle hypophysectomy effects on behavior. This contrast suggests that the effects of

quinpirole on checking/sensitization may be related to a change in dendritic morphology that is quite specific. In particular, a Drug effect was the main statistical outcome of behavioral analyses, and a Drug effect on branching in Cg3 was a unique outcome of statistical analyses of dendrite morphology. Hence, the most pertinent of the morphological dendritic changes for sensitized locomotion and compulsive checking, may be an increase in apical branching in Cg3, a plausibility congruent with the mPFC receiving extensive dopaminergic innervation (Lindvall & Bjorklund, 1987).

Quinpirole changed other aspects of dendrite morphology as well, with the change in spine density being most prominent. Alterations in spine density are a well-established effect of chronic treatment with dopaminergic stimulants, such as amphetamine and cocaine (Robinson & Kolb, 2004). It may seem surprising however that chronic quinpirole treatment produced effects opposite from those of amphetamine and cocaine. As shown in the present study, quinpirole decreased and increased spine density on dendrites in Cg3 and Par1, respectively. In contrast, as reviewed by Robinson and Kolb (2004), amphetamine increased spine density of apical Cg3 dendrites, produced no change in basilar Cg3 dendrites, and decreased spine density in both apical and basilar Par1 dendrites; cocaine produced an increase in spine density of Cg3 apical and basilar dendrites. A plausible explanation for such opposite profiles is elaborated below.

The differential drug effects may be related to the opposite ways that amphetamine/cocaine and quinpirole affect dopamine release, producing, respectively, increased and decreased levels of extracellular dopamine (Imperato *et al.,* 1988; Linthorst *et al.,* 1991; Rouge-Pont *et al.,* 2002; Koeltzow *et al.,* 2003). Strong homeostatic mechanisms exist to regulate and maintain a stable level of dopamine activity, through biochemical processes that adjust the concentration of dopamine released and the sensitivity of dopamine receptors (Cooper *et al.,* 2003). Moreover,

dopamine neurons show also structural adjustments to effectively normalize DA receptor stimulation by increasing terminal arbor size when dopamine levels are low and pruning terminal contacts when dopamine levels are high (Parish *et al.,* 2001; Parish *et al.,* 2002b; Parish *et al.,* 2005a). The mechanisms for this terminal arbor remodeling appears to require D2 autoreceptors and the dopamine transporter (Stanic *et al.,* 2003; Parish *et al.,* 2005b), and may also involve astroglia, microglia, and cytokines (Parish *et al.,* 2002a). Accordingly, the opposite effects of amphetamine/cocaine and quinpirole on spine density may reflect different compensatory regulation of axonal arbor and synaptic contacts, secondary to the drugs' effects on extracellular dopamine levels.

It should be noted that because both amphetamine/cocaine and quinpirole lead to stimulation of D2/D3 autoreceptors that may reduce terminal arbor size (Parish *et al.,* 2001), the drugs' opposite effects on spine density must involve mechanism(s) beyond activation of pre-synaptic autoreceptors. As suggested by the authors (Parish *et al.,* 2005a), the nature of plastic changes in the post-synaptic targets may be influenced also by synaptic dopamine: High levels of extracellular dopamine (as under amphetamine/cocaine) may affect contact formation possibly by direct D1 receptor stimulation; low synaptic dopamine (as under quinpirole) may have a different effect on contact formation because of minimal stimulation of D1 receptors.

Not only different drugs, but also one and the same drug may produce opposite effects on spine density. With chronic quinpirole treatment, spine density rose in Par1, a brain region with low dopamine (Descarries *et al.,* 1987) and declined in Cg3, an area with relatively high dopamine (Lindvall & Bjorklund, 1987; Descarries *et al.,* 1987). An interesting speculation that could account for the spine density findings postulates the presence of regional differences in the arrangement of dopaminergic innervation, with a preponderance of direct dopamine innervation

of target dendrites in one region and a preponderance of indirect innervation in the other area. In the condition involving direct innervation, a downregulation of spine density may be expected to limit quinpirole-induced hyper-stimulation of post-synaptic dopamine receptors on target dendrites. In the condition involving indirect innervation of the target dendrites through an inter-neuron, quinpirole hyperstimulation may be expected to induce primary changes in the inter-neuron and thus resulting in opposite compensatory adjustments in spine density on the target dendrites. The present results do not provide evidence as to which neuroanatomical arrangement is more typical of Cg3 or Par1 because specific predictions depend on knowledge whether the particular connections provide excitatory or inhibitory inputs to the target dendrites.

Conclusion

Even though in the absence of pituitary axis hormones there can be a substantial development of locomotor sensitization (Culver & Szechtman, 2004), the present findings indicate that this development is limited, not reaching full expression. Nevertheless, the expression of quinpirole-induced compulsive checking is full-blown in hypophysectomized animals, with one measure suggesting even greater compulsiveness. Compulsive checking may be coupled more closely to drug-induced increases in branching of apical dendrites in Cg3 than to quinpirole-induced changes in spine density of dendrites in Cg3 and Par1. It is suggested that changes in spine density may reflect compensatory adjustment mechanisms in dopamine-innervated regions and may depend on psychostimulant drug effects on extracellular dopamine levels.

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Figure 1. Effect of hypophysectomy on the locomotor response to repeated injections of quinpirole (0.5 mg/kg). (**a**) Locomotor activity during a 55-min test in a large open field after each injection of saline (Sal) or quinpirole (QNP). (**b**) Locomotor activity during a 90-min test in activity chambers for injections 1 to 8. Rats (n=6/group) were first tested in activity chambers and then transferred for tests in the open field. The total distance traveled during the session is indicated on the left Y-axis while the right Y-axis indicates the total distance traveled divided by the duration of session (that is, average distance in meters per min). Asterisks indicate a significant difference from injection 1 ($p < 0.05$). Data for activity chambers was obtained from (Culver & Szechtman, 2004).

Figure 2. Effects of hypophysectomy on the spatial distribution of locomotor activity in a large open field during the course of six injections of saline or quinpirole. Top panel shows trajectories of locomotion during injections 1 to 6 in a representative rat from each group. The trajectories during the entire session are shown; each line represents a trajectory of locomotion. The locations of the four objects in the open field are represented by gray squares. Lower panel shows the mean values of the path stereotypy index (*left*) and 2 standard deviational ellipse (*right*) across six quinpirole or saline injections. Hypophysectomized rats treated with saline were particularly inconsistent across injections for the measure of 2 standard deviational ellipse. This variance is likely due to the performance of one rat that immediately settled near an object located close to the place of its introduction into the open field, a behavior that it showed only on injections 2 and 5. Legend. NS=normal rats injected with saline; NQ=normal rats injected with quinpirole; HS=hypophysectomized rats injected with saline; HQ=hypophysectomized rats injected with quinpirole. Asterisks indicate a significant difference from injection 1 ($p < 0.05$).

Figure 3. Time course of locomotor activity following injections of saline or quinpirole in

normal and hypophysectomized rats. Inset: Change in amount of locomotion across injections during two time periods (5 to 20 min after each injection *versus* 20 to 55 min after each injection). Solid lines represent a significant slope of linear regression; a dotted line indicates that the slope was not significant. Legend. NS=normal rats injected with saline; NQ=normal rats injected with quinpirole; HS=hypophysectomized rats injected with saline; HQ=hypophysectomized rats injected with quinpirole.

Figure 4. Effects of hypophysectomy on compulsive checking behavior in the quinpirole model of OCD. Measures of checking behavior are shown for six successive injections of quinpirole. Legend. NS=normal rats injected with saline; NQ=normal rats injected with quinpirole; HS=hypophysectomized rats injected with saline; HQ=hypophysectomized rats injected with quinpirole.

Figure 5. Effects of hypophysectomy and quinpirole treatment on dendrite morphology as measured by spine density (*left* column), dendritic branching (*middle* column) and lengths of dendrites (*right* column) in two brain regions (Cg3, medial prefrontal cortex; Par 1, somatosensory parietal cortex). Measurements for apical and basilar dendrites are presented separately. Values are the estimated marginal means and standard errors as obtained in the ANOVAs. Significant main effects of Hypophysectomy and of Drug Treatment are indicated by the symbols † and ‡, respectively; a significant interaction effect between Hypophysectomy and Drug Treatment is indicated by the letters HxD on the graph. Legend. NS=normal rats injected with saline; NQ=normal rats injected with quinpirole; HS=hypophysectomized rats injected with saline; HQ=hypophysectomized rats injected with quinpirole.

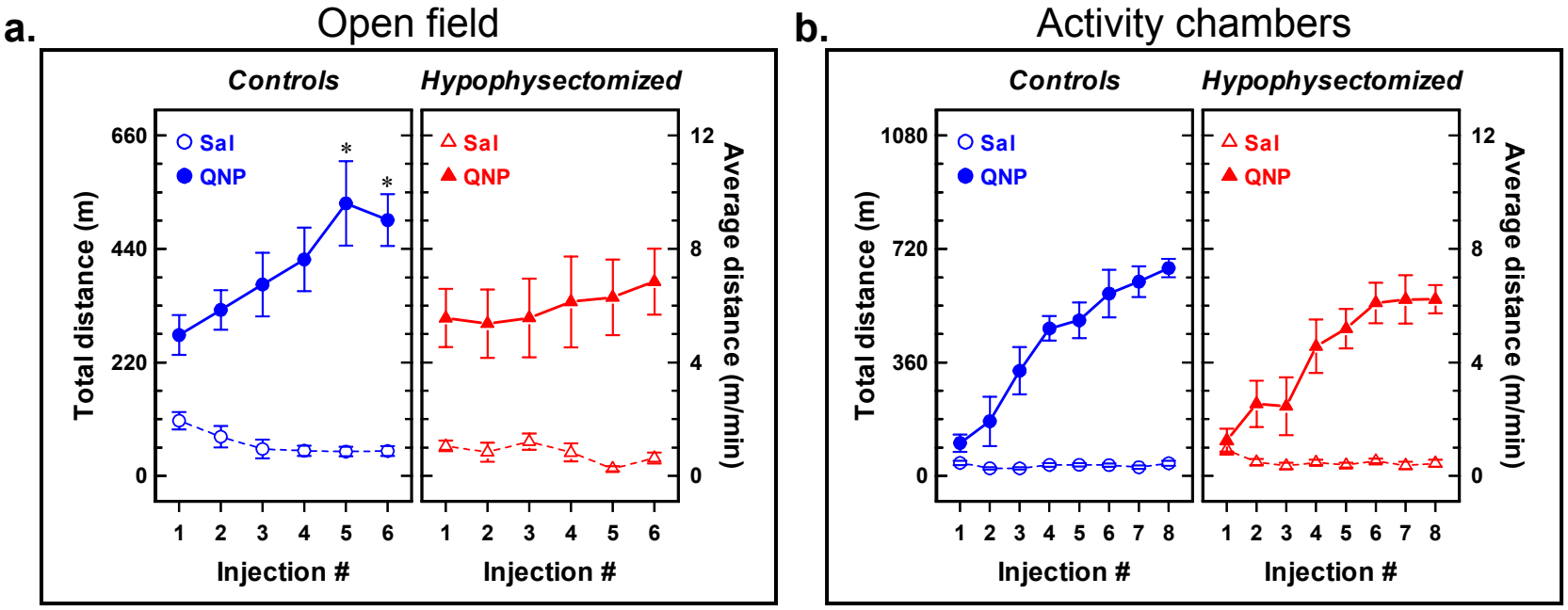

a.
Open field
Controls
Hypophysectomized
Sal
QNP
Total distance (m)
Average distance (m/min)
Injection #
660
440
220
0
12
8
4
1 2 3 4 5 6
*
b.
Activity chambers
1080
720
360
1 2 3 4 5 6 7 8

Fig. 1

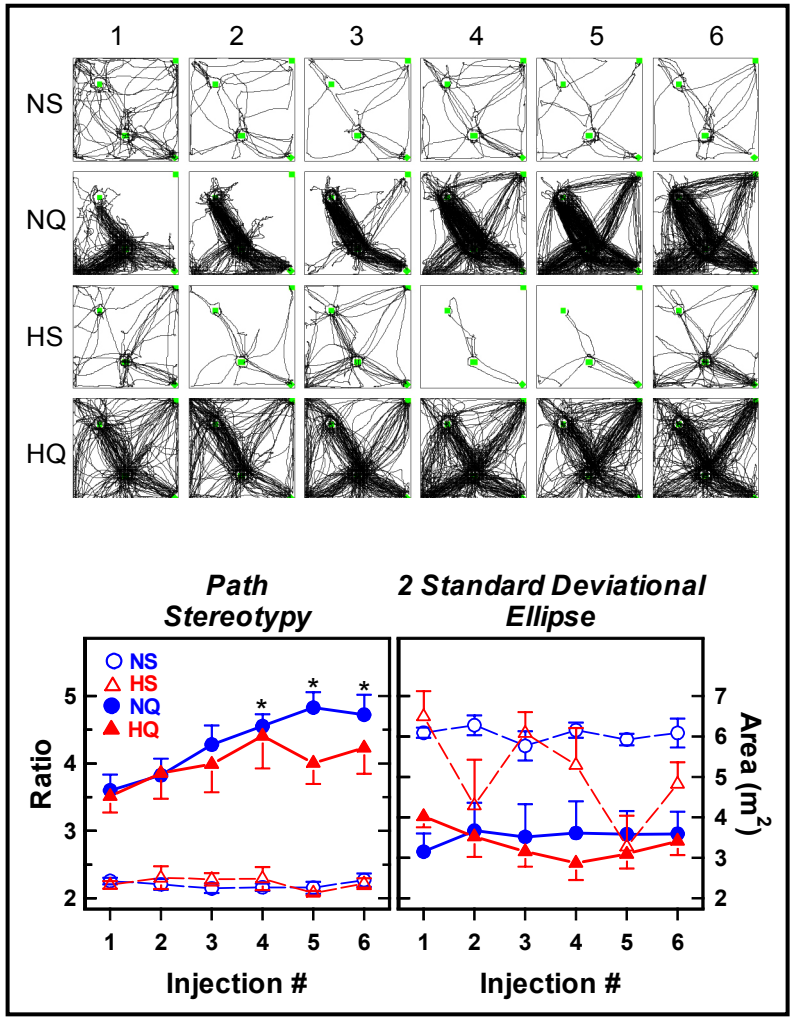

1
2
3
4
5
6
NS
NQ
HS
HQ
Path Stereotypy
2 Standard Deviational Ellipse
NS
HS
NQ
HQ
Ratio
Area (m2)
Injection #
Injection #

Fig. 2

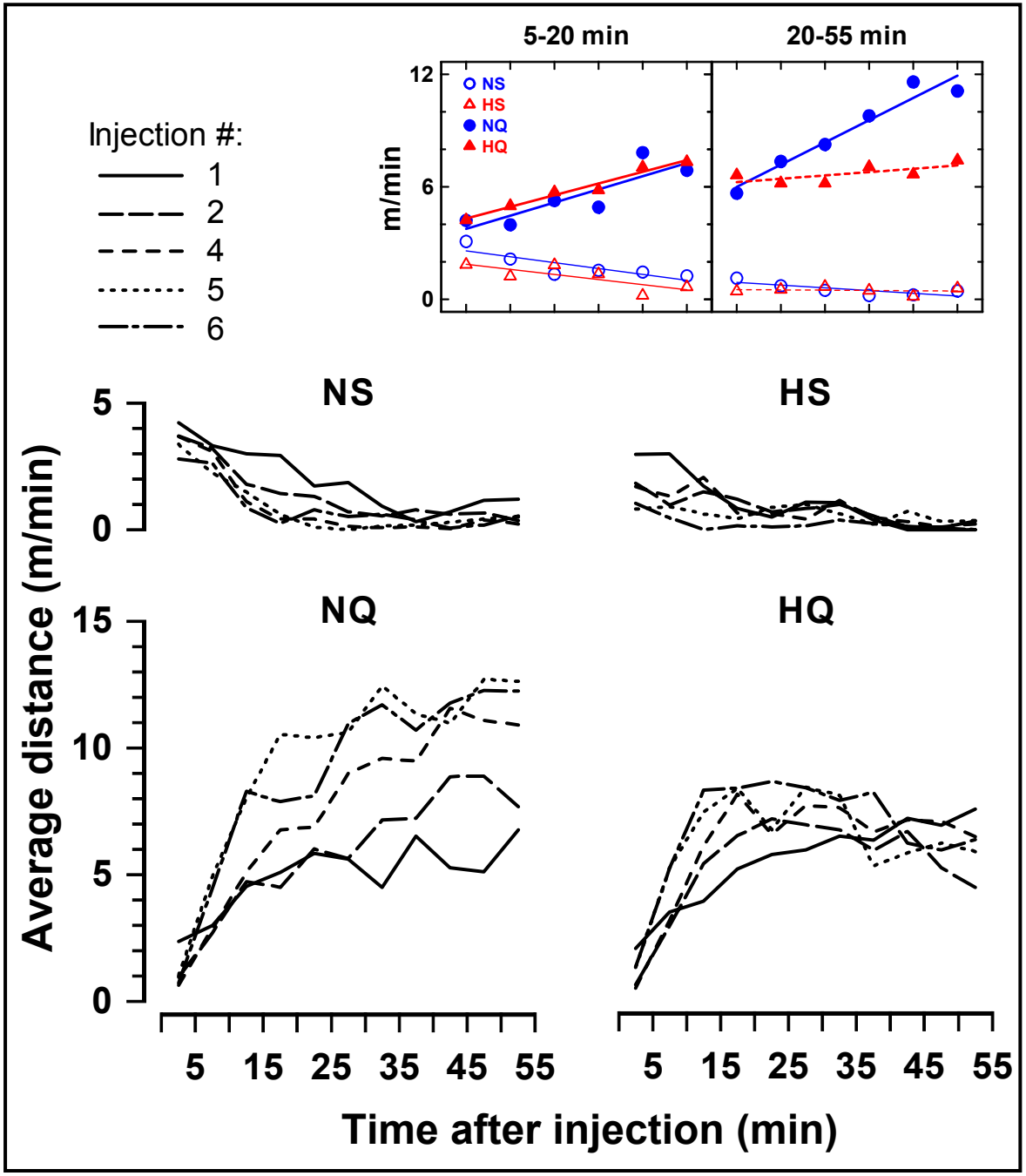
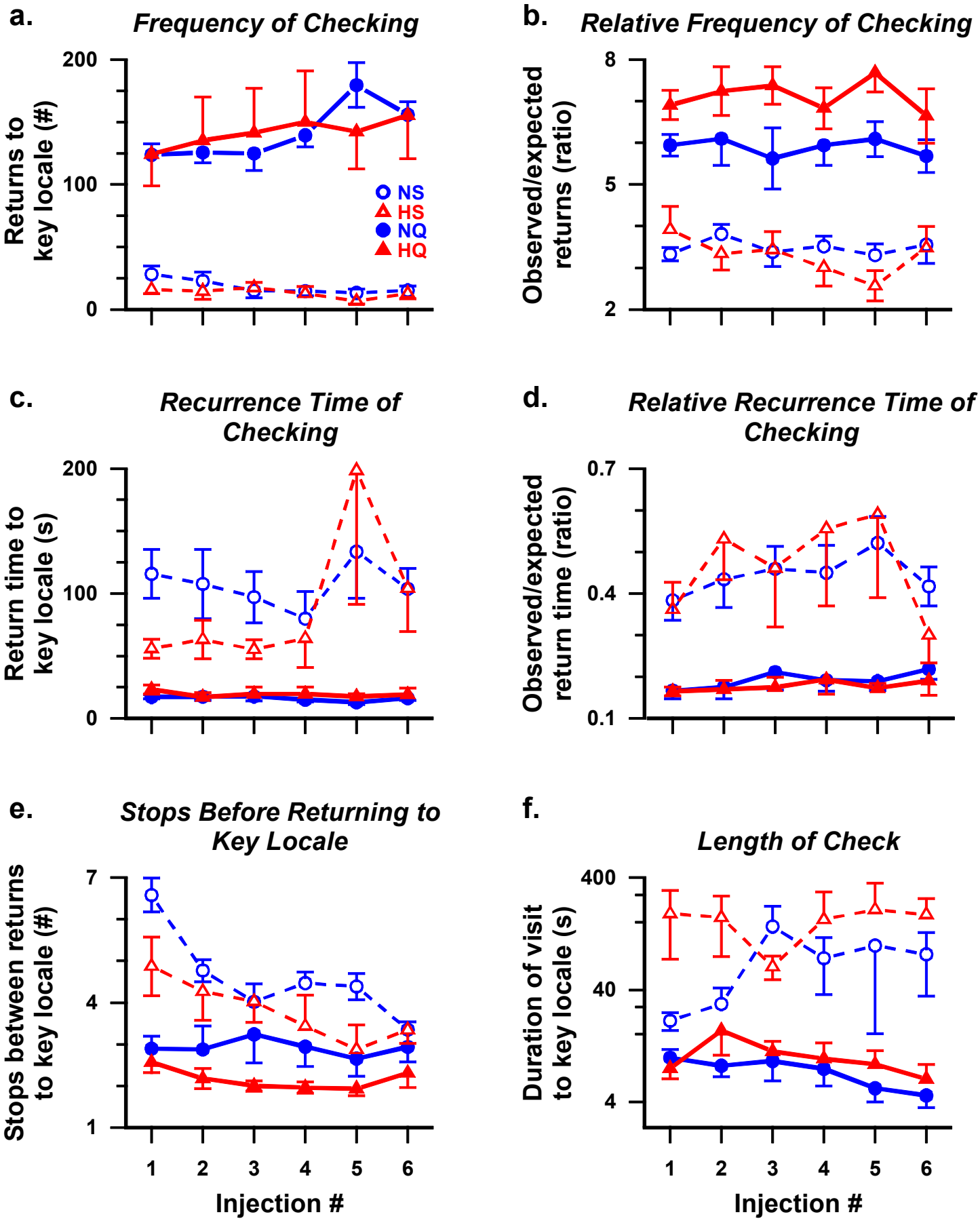

5-20 min
20-55 min
12
6
0
m/min
NS
HS
NQ
HQ
Injection #:
1
2
4
5
6
NS
HS
NQ
HQ
5
0
15
10
5
0
Average distance (m/min)
5 15 25 35 45 55
5 15 25 35 45 55
Time after injection (min)

Fig. 3


a.
Frequency of Checking
Returns to key locale (#)
200
100
0
NS
HS
NQ
HQ
b.
Relative Frequency of Checking
Observed/expected returns (ratio)
8
5
2
c.
Recurrence Time of Checking
Return time to key locale (s)
200
100
0
d.
Relative Recurrence Time of Checking
Observed/expected return time (ratio)
0.7
0.4
0.1
e.
Stops Before Returning to Key Locale
Stops between returns to key locale (#)
7
4
1
1 2 3 4 5 6
Injection #
f.
Length of Check
Duration of visit to key locale (s)
400
40
4
1 2 3 4 5 6
Injection #

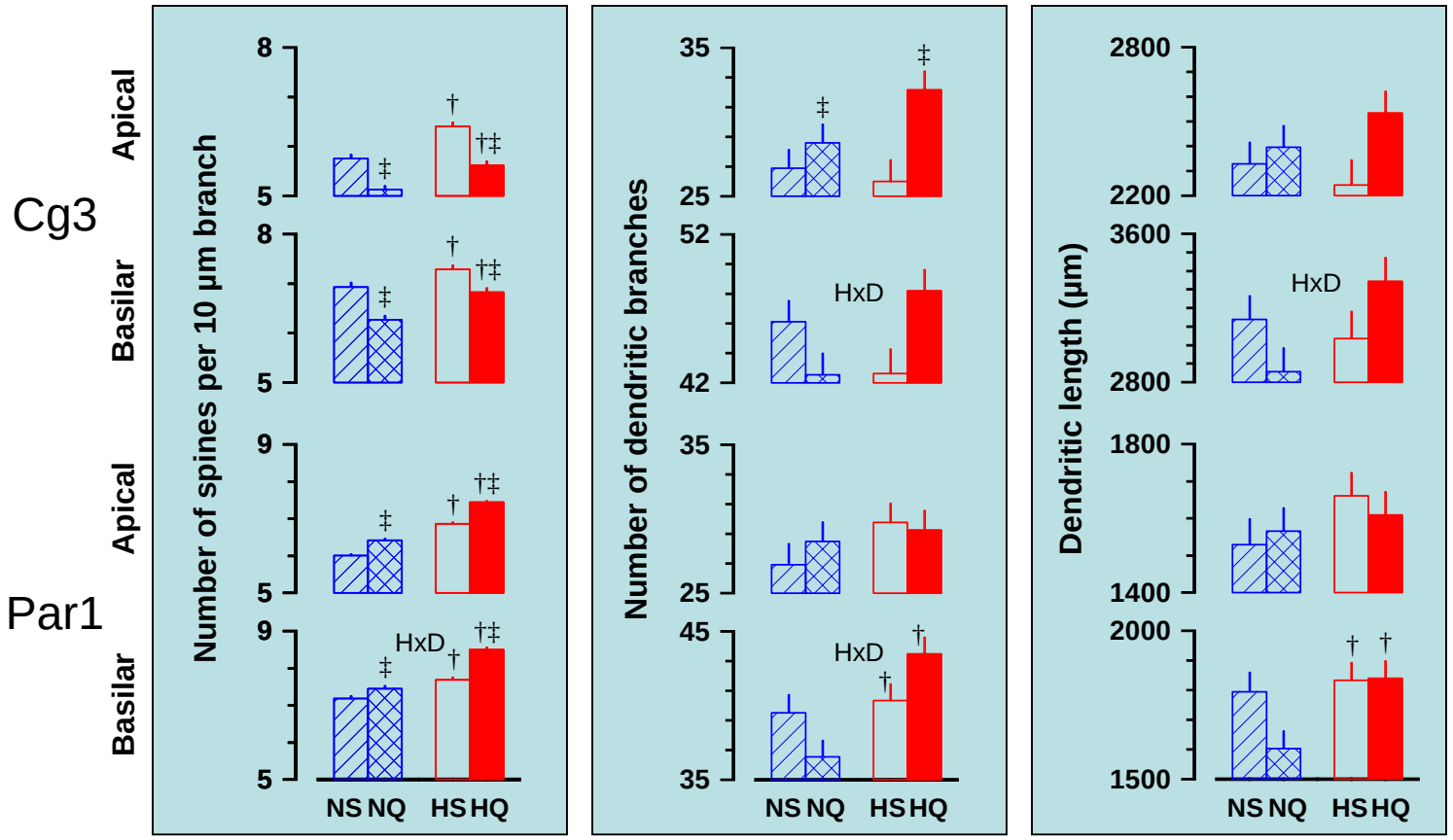

Cg3
Par1
Apical
Basilar
Apical
Basilar
Number of spines per 10 µm branch
Number of dendritic branches
Dendritic length (µm)
HxD
NS NQ HS HQ

Fig. 5

Table 1. Summary of statistical analyses for measures of compulsive checking shown in Figure 4. Each dependent variable was analyzed by a Hypophysectomy (H) by Drug Treatment (D) by Injection Number (I) ANOVA with repeated measures on the last factor.

		Frequency of checking		Relative frequency of checking		Return time		Relative return time		Stops before returning		Length of check	
Source	df	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Between-Subjects													
H	1	0.01	0.9	2.2	0.2	0.01	0.9	0.0	0.9	4.367	0.051	2.04	0.2
D	1	44.3	0.001	62.1	0.001	33.6	0.001	30.8	0.001	21.0	0.001	12.0	0.003
H * D	1	0.01	0.9	2.7	0.1	0.1	0.7	0.1	0.7	0.0	0.9	1.8	0.2
Error	20												
Within-Subjects													
I	5	1.52	0.2	0.8	0.5	3.06	0.013	2.27	0.054	8.9	0.001	0.6	0.7
I * H	5	1.72	0.1	1.05	0.4	1.55	0.2	0.99	0.4	2	0.080	1.1	0.4
I * D	1	4.58	0.001	2.42	0.042	3.53	0.006	2.26	0.055	5.6	0.001	0.7	0.6
I * H * D	5	0.98	0.4	1.52	0.2	1.47	0.2	0.67	0.6	4.8	0.001	1	0.4
Error(I)	90												