KAPPA-OPIOID RECEPTOR STIMULATION QUICKENS PATHOGENESIS OF COMPULSIVE CHECKING IN THE QUINPIROLE SENSITIZATION MODEL OF OBSESSIVE-COMPULSIVE DISORDER (OCD)

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Abstract

Repeated injections of the D2/D3 dopamine agonist, quinpirole, induces in rats locomotor sensitization and compulsive checking behavior, a phenomenon that may constitute an animal model of OCD. Considering that the co-joint treatment with quinpirole and the kappa opioid receptor agonist U69593 potentiates locomotor sensitization to quinpirole, the present study examined whether such co-stimulation also enhances compulsive checking and whether dopamine receptor supersensitivity mediates the augmentation effects. Results showed that co-treatment with U69593 had a robust accelerating effect on the acquisition of sensitized locomotion and compulsive checking but the effects on the expression of quinpirole sensitization were behavior-dependent, with the magnitude of locomotion elevated but not of compulsive checking. Quinpirole, and even U69593 that by itself did not induce sensitization, increased the proportion of dopamine D2 receptors in the high affinity state (D2High) in nucleus accumbens and striatum, indicating that elevation of D2High is not sufficient to account for sensitization or compulsive checking. It is considered that the animal model findings point to a potential role of kappa opioid systems in hastening the pathogenesis of OCD and that distinct brain regions may mediate the development and the expression of compulsive checking.

Keywords: kappa-opioid dopamine receptors co-activation, obsessive-compulsive disorder (OCD), animal model, quinpirole, U69593, components of sensitization, development of OCD, dopamine D2High affinity binding

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 (Brus, Kostrzewa, Nowak, Perry, & Kostrzewa, 2003; Mattingly, Rowlett, & Lovell, 1993; Szechtman, Talangbayan, Canaran, Dai, & Eilam, 1994b; Willner, Papp, Cheeta, & Muscat, 1992). 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 its behavior constitutes an animal model of obsessive-compulsive disorder (OCD)(Szechtman, Sulis, & Eilam, 1998). As reviewed elsewhere (Eilam & Szechtman, 2005; Joel, 2006; Korff & Harvey, 2006; Man, Hudson, Ashton, & Nutt, 2004; Szechtman & Eilam, 2005; Westenberg, Fineberg, & Denys, 2007), the argument for an OCD model stems from findings that the properties of the quinpirole preparation correspond to the essential properties of the human disorder, in at least 4 domains: (a) the spatiotemporal structure of compulsive checking in the quinpirole rat and in the OCD patient may be similar (Ben Pazi, Szechtman, & Eilam, 2001; Dvorkin, Perreault, & Szechtman, 2006; Eilam, Zor, Szechtman, & Hermesh, 2006; Szechtman, Sulis, & Eilam, 1998; Zor, Hermesh, Szechtman, & Eilam, 2007), (b) the motivational basis of checking behavior in the rat and the human patient may be the same (Boyer & Lienard, 2006; Feygin, Swain, & Leckman, 2006; Szechtman, Sulis, & Eilam, 1998; Szechtman & Woody, 2004; Whishaw, Gharbawie, Clark, & Lehmann, 2006; Woody & Szechtman, 2005), (c) modulation by external stimuli of quinpirole behavior and OCD compulsions appears to be equivalent (Ben Pazi, Szechtman, & Eilam, 2001; Szechtman et al., 2001; Szechtman & Eilam, 2005; Szechtman, Sulis, & Eilam, 1998; Zadicario, Ronen, & Eilam, 2007), and, (d) pharmacological treatment of

quinpirole-induced behavior shows some correspondence to pharmacotherapy of OCD (Brown et al., 2004; Lundberg, Carlsson, Norfeldt, & Carlsson, 2004; Martin, 2001; Salin-Pascual & Basanez-Villa, 2003; Szechtman, Sulis, & Eilam, 1998; Tizabi, Louis, Taylor, Waxman, Culver, & Szechtman, 2002; Ulloa, Nicolini, & Fernandez-Guasti, 2004).

However, while there exists a fairly strong body of evidence to indicate that the compulsive checking of quinpirole sensitized rats possesses the attributes of compulsive behavior in OCD patients, there is little available evidence for the associated claim that quinpirole-induced compulsive checking is dependent on a drug-induced sensitized state (Eilam & Szechtman, 2005; Szechtman, Culver, & Eilam, 1999; Szechtman, Sulis, & Eilam, 1998). The available evidence consists of two sets of findings: (a) development of sensitization and of full-blown compulsive checking follow a similar time-course (Dvorkin, Perreault, & Szechtman, 2006; Szechtman, Dai, Mustafa, Einat, & Sullivan, 1994a; Szechtman et al., 1994b), and, (b) expression of locomotor sensitization (e.g., Einat, Einat, Allan, Talangbayan, Tsafnat, & Szechtman, 1996; Szechtman, Talangbayan, & Eilam, 1993; Szumlinski, Allan, Talangbayan, Tracey, & Szechtman, 1997) is context-dependent as is the expression of compulsive checking (Szechtman, Sulis, & Eilam, 1998). The claim for a sensitization factor in compulsive checking would be strengthened by findings that manipulations which potentiate/attenuate sensitization exert corresponding effects on quinpirole-induced compulsive checking. In this regard, a recent study showed that co-treatment with the kappa opioid agonist U69593 markedly potentiated locomotor sensitization to quinpirole (Perreault, Graham, Bisnaire, Simms, Hayton, & Szechtman, 2006). This potentiation was observed in rats tested in small activity chambers, an environment which is not conducive for the display of compulsive checking. Therefore, the present study examined whether in the appropriate environment (a large open field) kappa

receptor co-stimulation would potentiate not only locomotor sensitization to quinpirole but also quinpirole-induced compulsive checking.

The study had also a second aim. It has recently been shown that a great variety of treatments that induce sensitization produce also an elevation in the proportion of dopamine D2 receptors in the high affinity state ($D2^{High}$); moreover, the authors proposed that this elevation in $D2^{High}$ may be a final common pathway that underlies the development of psychosis (Seeman et al., 2005; Seeman et al., 2006). Considering that sensitization seems also relevant in the development of OCD (at least in the quinpirole model), the present study evaluated whether the potentiation of sensitization by kappa agonist co-administration is similarly accompanied by a corresponding enhancement of $D2^{High}$.

Methods and Materials

Subjects

Forty experimentally naive male Long-Evans rats (Charles River, Canada), weighing 250-300 g at the start of the experiment were used. Rats were housed individually in polyethylene cages (35x30x16 cm) lined with Tek-Fresh Laboratory bedding made from 100% reclaimed virgin wood pulp (Harlan Teklad, Madison, WI) in a temperature controlled (22ºC) colony room, maintained on a 12 hour light-dark cycle (lights on at 07:00), and with free access to food and water. Following arrival rats were allowed to acclimatize to the colony room for 1 week and were then handled for 2 min daily for 5 days before the start of the experiment. All treatments were performed during the light phase of the day-night cycle. 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).

Drugs

All chemicals and reagents were obtained from Sigma Aldrich. QNP hydrochloride was dissolved in physiological saline and administered at a dose of 0.5 mg/kg. U69593 ((+)-(5a, 7a, 8ß)-N-methyl-–[7-(1-pyrrolidinyl)-1-oxaspirol[4.5]dec-8-yl]-benzeneacetamide was dissolved in sterile water containing 20% propylene glycol and administered at a dose of 0.3 mg/kg. All drug doses were administered at a volume of 1.0 ml/kg and injected subcutaneously under the nape of the neck. When two drugs were co-administered, U69593 was injected first, followed immediately by QNP; for non-drug injections, an equivalent volume of 20% propylene glycol vehicle and/or saline was administered. To induce sensitization, drugs were administered on a schedule of two injections per week (that is, every 3-4 days) for a total of 10 injections. This regimen was chosen because the effects of chronic treatment with quinpirole reach a plateau after 8 to 10 drug injections administered 2 to 8 days apart (Szechtman et al., 1994a; Szechtman et al., 1994b). For U69593, a dose of 0.3 mg/kg was chosen as this dose had been shown previously to produce significant changes in quinpirole-induced locomotor activity (Acri, Thompson, & Shippenberg, 2001; Perreault et al., 2006) .

Apparatus

The testing environment was a large open field consisting of a blue corinthine-top table (160 x 160 and 60 cm high) described previously (Dvorkin, Culver, & Szechtman, 2006), placed at least 70 cm from the walls and located in a testing room illuminated by fluorescent ceiling lights. Four small boxes 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. Three of the objects consisted of Plexiglas cubes (8 x 8 x 8 cm); two clear and one black, all of which had the tops painted blue to match the table and contained weights to secure the objects to the table. All of the cubes had one side open to allow the animal to put its head and front paws inside the box. The

fourth object was a rectangular glass container (10.5 x 8.5 x 7 cm) with the top side open but covered with a wire mesh and secured to the table with translucent caulking. The open field platform was subdivided virtually 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 antibacterial cleaner (Lysol). Two testing rooms were used, each with two identical open fields that were visually isolated from each other (and the operator) by curtains. 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 3.0 (Noldus Information Technology bv, Netherlands) system (Noldus, Spink, & Tegelenbosch, 2001; Spink, Tegelenbosch, Buma, & Noldus, 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.

Design and Procedure

To examine the effects of U69593 on sensitization to QNP and dopamine receptor binding, four groups were used: the experimental group (N=10) was treated chronically with both U69593 (0.3 mg/kg) and QNP (0.5 mg/kg), while the three control groups were similarly treated with either vehicle and QNP (N=10), U69593 and saline (N=10), or vehicle and saline (N=10). Rats were assigned into groups at random. On the day of testing, animals were weighed, transported in their home cage to an adjoining non-colony testing room, administered the appropriate injections, and placed immediately on the open field table where their activity was monitored for 55 min. Each animal was tested throughout the study at its assigned time of day

and open field. After each use, the open fields were thoroughly cleaned with Lysol diluted with water.

The treatment regimen consisted of 10 injections to monitor the development of sensitization and compulsive checking and was followed by the Object Rotation Test on Injection 11 to assess whether checking behavior is influenced by changes in the environment; for this test, the four objects in the open field were rotated in space by 180 degrees and thus moved to different open field locales (Szechtman, Sulis, & Eilam, 1998). While the Object Rotation Test is normally the last test in a study evaluating the presence of compulsive checking, in the present experiment there were 4 extra injections (unrelated to the study question) before rats were sacrificed for receptor binding assays. Thus, the experiential history of the groups before sacrifice included the following: a test on Injection 12 with normal treatment and objects returned to the same position as for injections 1 to 10; a test on Injection 13 where all groups received an injection of vehicle/saline to probe for conditioned effects; and tests with normal treatments on Injections 14 and 15. Results of behavioral analysis for injections 1 to 11 and injection 15 are presented in the present report; Injections 12 and 14 were not analyzed as they merely serve to re-introduce the usual test condition following a previous manipulation; findings from Injection 13 are not presented as they are not directly relevant to the question of quinpirole-induced compulsive checking, and furthermore showed no statistical difference between groups on any measure. Four days after the final injection (Injection 15), rats were sacrificed by decapitation and the brains were removed. Striatal and nucleus accumbens tissues were dissected, flash frozen, and immediately stored at -70ºC until used.

Dopamine Receptors Binding Assays

[^{3}H]Ligands. [^{3}H]Raclopride (60–80 Ci mmol) was obtained from PerkinElmer Life Sciences (Boston). [^{3}H]Domperidone was custom synthesized as [phenyl-3H(*N*)]domperidone (42 Ci mmol) by PerkinElmer Life Sciences, and used at a final concentration of 1.2–3 nM for competition with dopamine. The competition of [^{3}H]SCH23390 and dopamine was used to measure the proportion of $D1^{High}$ receptors. [^{3}H]SCH23390 (75-85 Ci/mmol) was obtained from PerkinElmer Life Sciences.

Saturation of dopamine D2 receptors by [^{3}H]raclopride (Scatchard analysis). The frozen tissue was blotted and weighed frozen. Buffer was added (50 mM Tris HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM NaCl) to yield 4 mg of tissue per ml. The method for determining the density of D2 receptors has been reported (Seeman, Tallerico, & Ko, 2003; Seeman, Tallerico, & Ko, 2004; Seeman, Tallerico, Ko, Tenn, & Kapur, 2002). Nonspecific binding for dopamine D2 receptors was defined as that in the presence of 10 μM *S*-sulpiride. The density of [^{3}H]raclopride binding sites and the dissociation constant (*K*d) were obtained by Scatchard analysis.

Competition between dopamine and [^{3}H]raclopride or [^{3}H]domperidone. The competition between dopamine and [^{3}H]raclopride or [^{3}H]domperidone for binding at the dopamine D2 receptors was done as reported (Seeman, Tallerico, & Ko, 2003). The competition data were analyzed by using a program that provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit (Seeman, Tallerico, & Ko, 2004).

Competition between dopamine and [^{3}H]raclopride or [^{3}H]domperidone for the proportion of dopamine D2/3^{High} receptors, and between dopamine and [^{3}H]SCH23390 for the proportion of D1^{High} receptors. To measure the proportion of dopamine receptors in the high-

affinity state for dopamine, the competition was measured between dopamine and [^{3}H]raclopride or [^{3}H]domperidone for dopamine D2/3^{High} receptors, and between dopamine and [^{3}H]SCH23390 for the proportion of D1^{High} receptors (Seeman, Ko, Willeit, McCormick, & Ginovart, 2005). More specifically, the total proportion of D2/3^{High} receptors was defined as that component of [^{3}H]raclopride or [^{3}H]domperidone binding that was inhibited by dopamine in the high-affinity concentration range with the inhibitory plateau generally occurring between 10 and 350 nM dopamine. The proportion of D2^{High} receptors was defined as that component of [^{3}H]raclopride or [^{3}H]domperidone binding that was inhibited by dopamine (in the high-affinity concentration range) in the presence of either 15 nM pramipexole or 1 μM U99194 (Seeman, Wilson, Gmeiner, & Kapur, 2006). The proportion of D3^{High} receptors was defined as the difference between the measurements for D2/3^{High} and D2^{High}.

Data Analysis

Locomotion in the open field. Two aspects of locomotor activity in the open field were quantified: distance traveled and the 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 3.0 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, Culver, & Szechtman, 2006) and were followed here. Total distance traveled was calculated as the sum of distances moved in progression segments and lingering episodes. For measures of spatial distribution, the smoothed *x,y* coordinates were rounded up to the nearest centimeter values, effectively superimposing on the open field a grid density of 1 cm by 1 cm

squares. As described in detail (Dvorkin, Culver, & Szechtman, 2006), *path stereotypy ratio*, which reflects the relative frequency of repetitions of travel along the same paths, is the number of grid squares that the rat passed through twice or more times divided into the total number of times that the rat passed through these grids. Similarly (Dvorkin, Culver, & Szechtman, 2006), *area of 2 standard deviational ellipse (2SDE)* (Lefever, 1926), which reflects the extent of the space covered by the trajectories of locomotion, is the area of the ellipse surrounding 95% of the trajectories (squares passed through at least once) and was computed using CrimeStat III spatial statistics program (Levine, 2004). In the present study, two additional measures of spatial distribution are introduced: *density of paths*, and, *relative focus on key locale*. The former measure represents the percentage of the total open field area occupied by the trajectories of locomotion, and is the total number of 1 cm by 1 cm grids traversed at least once divided by the total number of 1 cm by 1 cm squares in the open field (expressed as a percentage). The latter measure reflects the diversity of locales the rat visited in the open field, and is the number of visits to the most visited locale divided by the total number of visits to all locales in the open field (expressed as a percentage).

Checking behavior. Using a virtual implementation of the coordinate system of 25 open field locales (places) (Szechtman, Sulis, & Eilam, 1998), the EthoVision trackfiles were processed as described previously (Dvorkin, Perreault, & Szechtman, 2006), and the frequency of visits and the duration of stops in each locale were computed accordingly (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, Sulis, & Eilam, 1998), and generally for untreated rats (Eilam & Golani, 1989), the

locale with most visits is also the locale with longest duration of stops. Checking behavior was originally defined with reference to the most visited locale (Szechtman, Sulis, & Eilam, 1998) but in subsequent studies it was measured with reference to the locale with the maximal cumulative duration of stops (Dvorkin, Perreault, & Szechtman, 2006; Szechtman et al., 2001; Tizabi et al., 2002). The latter approach was adapted because it was easier to implement computationally and fitted the definition of a home base (according to Eilam & Golani (1989), the rat's home base can be identified as the place with the maximal duration of stops). In the present study, we revert to the original approach of measuring checking behavior with reference to the most visited place (called here, *key locale*) because computational difficulty is no longer a practical problem and because this approach does not assume *apriori* that the locale of interest is the home base. Nevertheless, as noted, the two approaches almost always provide identical results. Discrepancies between the two approaches may occur when it is difficult to unambiguously identify the reference locale because the most visited locale and the locale with maximal duration of stops are different spots but with nearly identical number of visits (that is, they differ by 3 or fewer visits). In those instances, choosing the alternate place as the key locale may reduce substantially extreme values for some of the parameters of checking behavior and hence error variance. Therefore, in the present study, the key locale was, by default, the place with the highest number of visits. However, in the situation when there was a difference of 3 or fewer visits between the locale with highest ranked visits and the locale with highest ranked duration of stops, then the locale with the longer duration of stops was used as the key place.

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 (Dvorkin, Perreault, & Szechtman, 2006; Szechtman et al., 2001; Szechtman, Sulis, & Eilam, 1998; Tizabi et al., 2002). As reviewed elsewhere (Eilam & Szechtman, 2005; Szechtman & Eilam, 2005) and summarized in the Introduction, 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 in the open field (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 (Ben Pazi, Szechtman, & Eilam, 2001; Szechtman, Sulis, & Eilam, 1998).

Criteria for compulsive checking (Szechtman, Sulis, & Eilam, 1998) involve not only the above measures but also an Object Rotation Test to probe that quinpirole-induced behavior is not

an automatism but is coupled appropriately to environmental context, as would be expected if the animal were engaged in checking. In the Object Rotation Test, the four objects are moved from their usual location in the open field into a new position by virtue of rotating the arrangement of objects in space by 180 degrees. The expectation is that the animal will shift its focus to new object/locales and visit less often the previous places of interest (Szechtman, Sulis, & Eilam, 1998). Although in the original formulation (Szechtman, Sulis, & Eilam, 1998) no prediction was made as to the resultant changes in the actual parameters of checking behavior, nevertheless, some attenuation of checking behavior should be expected, given that the usual environment is re-arranged. Measures of checking behavior on the Object Rotation Test were computed as above, and compared to performance on injection 10 using paired *t*-tests.

Hyperbolic function fitted to sensitization data. A hyperbolic equation was used to describe the development of sensitization to quinpirole. A nonlinear curve-fitting algorithm (Fig.P Version 2.98, Fig.P Software Corporation, Hamilton, ON) was used to estimate the best fit parameters for the following asymmetric sigmoid equation:

$$R = R_{min} + (R_{max}-R_{min}) / (1 + ((X/X_{50})^{-n}))$$

where X is the number of injections (I) and R is the response after I number of quinpirole injections. The estimated parameters are: R_{max}, the maximal response after an infinite number of injections; X_{50} (I_{50}), the number of injections to reach the half-maximum response; n, some power coefficient that represents the sigmoidicity of the function; and, R_{min} which is the lowest response and which was set fixed at the lowest value observed (usually the value at injection 1). For development of sensitization, I_{50} and R_{max} are taken as measures of the speed of sensitization and the maximum capacity attained, respectively. The use of this function for sensitization to

quinpirole was described previously (Perreault et al., 2006; Szechtman et al., 1994b; Szumlinski et al., 1997).

Results

As locomotor sensitization invariably accompanies the development of quinpirole-induced compulsive checking (Eilam & Szechtman, 2005), we present in the section below the findings related to locomotor activity and consider in the subsequent sections the findings specific to checking behavior and neurochemistry.

Effects of U69593 Co-treatment on Locomotion and Locomotor Trajectories

Development of locomotor sensitization. Figure 1 presents the profile of locomotor sensitization exhibited by rats treated with quinpirole-alone (VQ group) as well as by those co-treated with quinpirole and the kappa agonist U69593 (UQ group): Bar graphs indicate group means at each injection while lines represent the performance of each individual rat, ranked from lowest to highest by mean distance traveled during the course of 10 injections. Inspection of the group means (bars) suggests the expected presence of locomotor sensitization in the VQ group and enhancement of sensitization in the UQ animals such that by injection 10 the co-treated rats traveled almost twice as much as the animals treated with quinpirole-alone. Statistical analyses support these observations as shown in Tables 1 and 2. Furthermore, as is evident in Figure 1, there was no locomotor sensitization in the control groups, neither the vehicle-saline controls (VS group) nor in the kappa-alone control rats (US group).

Inspection of locomotor performance shown by individual rats (Figure 1, lines) reveals that even though the US group showed no locomotor sensitization, one of the rats treated with U69593-alone showed an extremely high level of locomotion, on a par with the amount shown by quinpirole-treated rats. The atypical response of this animal (rat US17) is of interest here

because the high level of locomotion may illuminate whether mere hyperactivity necessarily results in compulsive checking behavior. As will be shown later in the section on checking behavior, the data suggests that the hyperactivity of rat US17 was not accompanied by development of compulsive checking. To lay the groundwork for the presentation of this data, Figure 2 displays the trajectories of locomotion shown by rat US17 following each injection of U69593, as well as the trajectories of locomotion for a selected rat from each of the other three groups. These rats were selected for the closest available match to rat US17 in terms of mean distance traveled across 10 injections (for US17 = 4.40 m/min, VQ32 = 4.38 m/min, UQ09 = 4.42 m/min, VS33 = 1.43 m/min; the selected rats are identified in Figure 1 by a line with circle symbols). The paths of locomotion shown in Figure 2 (top) provide also a context for the set of results presented next, namely, an analysis of the spatial distribution of locomotor activity in the open field environment.

Morphology of locomotor trajectories. Inspection of locomotor trajectories shown in Figure 2 provides a visual impression that rats from different groups locomoted in the open field along distinctly different paths, each rat laying its own unique network of trajectories forming a characteristic spatial structure or morphology. To describe quantitatively this morphology of locomotor routes and assess the effects of drug treatments on it, Figure 3 compares the groups on four variables that measure different aspects of the spatial distribution of locomotor activity, as discussed below.

Figure 3a shows for each group the profile of change during the course of chronic treatment in *path stereotypy ratio*, an index of repetitive travel along the same path and thus a measure of the tendency for locomotor trajectories to form common routes. Three findings are immediately apparent: (1) rats receiving quinpirole (with or without U69593 co-treatment)

showed greater path stereotypy than the two control groups, indicating that quinpirole-injected rats confined their locomotion in space to repeated travel along the same paths more than controls which, on the average, traveled along a route only twice (compared to 3-6 times for rats receiving quinpirole); (2) the magnitude of repeated travel along the same path increased across injections in the VQ and UQ groups, that is, path stereotypy showed sensitization; and, finally, (3) co-treatment with the kappa agonist potentiated quinpirole-induced sensitization in terms of the magnitude attained (R_{max} increased from a path stereotypy ratio of 4.6 to 5.9) and the rate of sensitization (I_{50} dropped from 5.8 to 2.4 injections), as summarized in Table 1.

Figure 3b displays for each group a measure of spatial dispersion of locomotor trajectories in the open field. As can be seen from an inspection of the trajectories in Figure 2, control rats locomoted along the edges of the open field but both of the rats treated with quinpirole showed relatively little travel along edges, suggesting a spatially more confined spread of locomotor trajectories under quinpirole. A quantitative measure of the extent of the area over which the trajectories of locomotion are dispersed is given by the *area of 2 standard deviational ellipse (2SDE)*, shown in Figure 3b. As is evident by their significantly smaller values for 2SDE, both of the quinpirole-treated groups confined their locomotion to a smaller portion of the open field, acutely and throughout the course of drug treatment compared to controls ($p<.05$, Duncan multiple range test). Co-treatment with the kappa agonist resulted in an even tighter spatial confinement of locomotor trajectories, but only during injections 2 to 5 (for Group x Injection interaction, $F(27,324)=1.969$, $p=0.003$; followed by comparisons of estimated marginal means and 95% confidence intervals, Table 2).

Figure 3c provides yet another index of spatial dispersion, *density of paths*, an index related to the proportion of the open field area covered by locomotor trajectories. The physical

underpinnings of this index can be garnered from an inspection of the trajectories in Figure 2, and for illustrative purposes from a comparison of rats US17 and UQ09, say at injection 7. As can be seen, the trajectories of rat US17 cover virtually every part of the open field while those of rat UQ09 adjoin each other tightly forming major and distinct tracks ("highways"). Consequently, the percentage of total open field area occupied by the trajectories of rat US17 should be larger than the corresponding area encompassed by tracks of rat UQ09—indeed, those values at injections 7 for rats US17 and UQ09 are 48.9% and 25.5%, respectively. The corresponding values for rat VS33 and rat VQ32 are 14.6% and 45.3%; the observation that the lowest value for density of paths was in the control rat is related to the rat having the lowest amount of locomotion, imposing a lower value for the upper limit of path density (it should be noted that, in general, while the level of locomotion imposes an upper boundary on the value of path density, the actual value of the index can vary widely for the same level of activity as its value depends on the spatial dispersion of the trajectories; in essence, it is the proportion of the open field "painted black"). As can be seen in Figure 3c, the mean density of paths increased across injections in both the VQ and UQ groups, but remained fairly stable in the two control groups. Inspection of locomotor trajectories illustrated in Figure 2 suggests that the increase in path density across injections in the quinpirole-treated groups reflects the formation of wider well defined tracts rather than an increase in the number of paths dispersed across the open field. The mean density of paths across injections differed significantly between every group, including a significant difference between the US and the VS groups (Duncan multiple range test, $p<.05$), consistent with the examples illustrated in Figure 2. As shown in Table 1, the parameters defining the curve of paths density as a function of number of injections showed no statistical differences between the VQ and the UQ groups, suggesting that the effects of co-

treatment with the kappa agonist may be to elevate the amount of paths density by a similar amount from the first injection on.

Finally, Figure 3d shows a measure of spatial distribution, *relative focus on key place*, that may not be discernable from a mere visual inspection of locomotor trajectories but which quantifies the diversity of visited locales during travel in the open field. The variable *relative focus on key place* measures what proportion of total visits in the open field are concentrated on the most visited locale ("key place"); this proportion is an approximate index of spatial diversity of places visited because higher proportions suggest more visits to the same locale and fewer visits elsewhere to other locales. As can be seen in Figure 3d, during the course of repeated injections of quinpirole, the VQ group steadily increased the proportion of visits to the key locale such that by injection 10, more than 30% of all stops in the open field were at the same locale. In contrast, the UQ group attained an even higher concentration of visits to one locale (about 35%) already by injection 2 but this value declined to less than 30% by injections 8-10. These results suggest that VQ rats steadily restricted their visits to smaller numbers of locales during the course of chronic treatment; in contrast, UQ animals almost immediately focused on an even smaller number of places but with repeated treatment expanded the number of visited locales to levels reached by VQ rats at end of chronic treatment. This suggestion that co-treatment with the kappa agonist produced a rapid, but transient, very narrow focus on a few locales, is supported statistically by a significant difference between the VQ and UQ groups, but only during injections 2 to 5 (for Group x Injection interaction, $F(27,324)=3.257$, $p<0.001$; followed by comparisons of estimated marginal means and 95% confidence intervals, Table 2).

Compulsive Checking Behavior

Using the criteria enumerated in the Methods to identify compulsive checking behavior, Table 3 shows that both of the groups injected with quinpirole (VQ and UQ groups) displayed on injection 10 the expected changes indicative of compulsive checking. Moreover, as shown in Figure 4, their behavior showed the expected context-dependency in that the number of visits increased significantly when an object was moved to a new locale, though the decline in the number of visits to the corner locale from which the object was removed (Site 14) was not significant in the VQ group (as found previously; Szechtman, Sulis, & Eilam, 1998) but achieved statistical significance in the UQ group. However, the spatial distribution of routes on the Object Rotation Test changed in all rats to encompass the new arrangement of objects, as illustrated in the example of path plots for rats VQ32 and UQ09 (Figure 4, compare to Figure 2). Finally, as shown in Table 3, both groups had some disruption in measures of compulsive checking on injection 11 compared to injection 10, albeit the pattern of statistical effects was not identical in the VQ and UQ groups. It is not possible to ascertain whether those differences indicate some fundamental distinction in the compulsive behavior of the two groups or merely reflect small quantitative variations related to baseline differences at injection 10. Nevertheless, the results do show clearly that rats treated with quinpirole (alone or in combination with U69593) developed compulsive checking.

However, it is difficult to discern from the data in Table 3 whether kappa co-treatment attenuated, exacerbated, or had no effect on the intensity of compulsive checking. This is because for some measures of checking the UQ group lay closer to controls than the VQ group (suggesting an attenuation of compulsive checking) while for other measures the UQ group was further away from control values, suggesting a potentiation of checking behavior. A possible

resolution of these apparently contradictory results is provided upon examination of the 6 measures of checking behavior during the course of treatment, shown in Figure 5.

Inspection of Figure 5 strongly favors the suggestion that kappa agonist co-treatment potentiated compulsive checking because by injection 4 or earlier—and on every measure of checking behavior—UQ rats achieved values that VQ rats did not reach until about injection 10. This accelerated development of compulsive checking in UQ group is evident also by considering the first injection on which the VQ and UQ groups showed full-blown compulsive checking, as evidenced by a significant difference from saline controls on all measures. Using this signpost, VQ rats achieved full-blown compulsive checking by injection 6 but in UQ rats this was evident already at injection 2 (Table 2). Consistent with an accelerated development of compulsive checking is the observation that for 3 of the 6 measures of checking there was a significant difference between the UQ and VQ groups during injections 2-4 (Figure 5 and Table 2).

Nevertheless, by injection 10, as shown in Figure 5 (b,d,e), there was a reversal between UQ and VQ groups in the ranking of their means for three of the measures of checking behavior, albeit only one of those reversals was statistically significant (relative frequency of checking at injection 10, VQ > UQ, $p<.05$). Consequently, the question exists whether such reversals are consistent with the proposed accelerated development of compulsive checking. We suggest that they are, on the basis of a comparison of Figure 5b and Figure 3d. Specifically, the profile of change in Figure 5b is remarkably similar to the profile of change for "relative focus on key place" shown in Figure 3d, suggesting that the two figures tap into the operation of a related process. The variable in Figure 3d indexes the diversity of locales that the rat visits, with higher values indicating a focus of activity on fewer places. As noted previously, Figure 3d shows that

VQ rats steadily restricted their visits to smaller numbers of locales during the course of chronic treatment, but UQ animals focused at the outset on small numbers of locales and with repeated treatment increased the range of visited locales, until, ultimately, the range of focus was roughly equivalent in the two groups. Accordingly, Figure 5 can be interpreted as indicating confinement of checking behavior in UQ rats within a very limited portion of the environment at start of treatment but expansion of its scope with repeated injections. This perspective suggests that the peculiar attenuation in three measures of checking shown by UQ rats (Figure 5b,d,e) may reflect these measures' sensitivity to distance between places being checked – presumably relatively nearby at start of treatment but further apart with repeated injections. In other words, kappa agonist co-treatment appears to trigger an early onset of compulsive checking within a narrow space but continued co-treatment induces the spread of checking to encompass a similar spatial range as in rats treated with quinpirole-alone. Indeed, consistent with this suggestion, measures of checking in VQ and UQ rats were more similar to each other on injection 15 than injection 10 (Table 3), and the calculated maximal capacity for frequency of checking (*Rmax*) did not differ between VQ and UQ groups (Table 1).

Relation Between Hyperactivity and Compulsive Checking

Because compulsive checking is expressed by repeated travel to and from the locale/object of interest, increased locomotion is a necessary component of quinpirole-induced checking behavior. However, as illustrated in Figure 2, the mere presence of hyperactivity does not necessarily imply development of compulsive checking. Rat US17 from the US group depicted in Figure 2 was treated chronically with the kappa agonist and showed as much locomotion across injections as the two rats (VQ32 and UQ09) from VQ and UQ groups, and yet, it did not develop compulsive checking unlike the quinpirole-treated animals. This contrast

in compulsive behavior is discerned in the values of the 6 measures of checking behavior on injection 10 shown for each rat in the graph (Figure 2, left bottom panel). As is evident, the values of rat US17 did not reach the values of the quinpirole-treated animals, except for "relative return time" where its value was close to rat UQ09. Similarly, in terms of the morphology of locomotor trajectories (Figure 2, right bottom panel), the spatial structure of rat US17 did not reach the quantitative measures of rats treated with quinpirole.

Effects of U69593 Co-treatment on Proportion of Dopamine Receptors in High Affinity State

Figure 6 shows the proportion of dopamine receptors in the high affinity state in two brain regions. As is evident, for the proportion of $D2^{High}$, there were significant and equivalent increases (compared to VS controls) in all treatment groups in the nucleus accumbens as well as the striatum. Similarly, there were comparable increases in the proportion of $D3^{High}$ in US, VQ and UQ groups in the nucleus accumbens, while the increase in the striatum reached significance for US rats only. Finally, a significant increase in the proportion of D1-like receptors in the high affinity state was evident only for US rats in the nucleus accumbens. It should be noted that the outlier rat for locomotion in the US group (rat US17) was not unusual for dopamine binding as its values lay within the group range for measures of dopamine receptors.

Discussion

The present study strengthens the supposition that sensitization induced by the dopamine agonist quinpirole is an important factor in the quinpirole model of OCD compulsive checking (Eilam & Szechtman, 2005; Szechtman, Culver, & Eilam, 1999; Szechtman, Sulis, & Eilam, 1998). The importance of drug-induced sensitization for compulsive checking is evidenced by the finding that a manipulation known to enhance locomotor sensitization to quinpirole had also

a potentiating effect on quinpirole-induced checking behavior. Specifically, as for locomotor sensitization to quinpirole (Perreault et al., 2006), co-treatment with the kappa agonist U69593 augmented quinpirole-induced checking by reducing markedly (from 6 to 2) the number of drug injections needed to produce the full-blown syndrome. Interestingly, while kappa agonist co-treatment exerted two effects on locomotor sensitization to quinpirole—it increased both the speed and the magnitude of locomotor sensitization (Perreault et al., 2006)—it had only a single effect on compulsive checking, accelerating its development but without producing an increase in the magnitude of checking. Thus, kappa agonist coadministration appears to have a robust accelerating effect on the acquisition of sensitized responding to quinpirole but its effects on the expression of quinpirole sensitization are behavior-dependent, with locomotion being enhanced but not compulsive checking.

The temporal association for hastened compulsive checking with a significantly smaller 2SDE in UQ compared to VQ rats during injections 2 to 5 (Figure 3b), may indicate that there exists a cause-effect relationship between the two phenomena or that they are relatively independent processes loosely coupled in time. The present results do not illuminate the merits of either hypothesis.

The finding of a differential effect of kappa agonist cotreatment on development (and not expression) of compulsive checking suggests a selective influence of U69593 on the pathogenic process induced by quinpirole. Such a selective effect is consistent with a body of evidence implicating separate neural systems mediating the development and the expression of drug-induced locomotor sensitization. In particular, there is evidence that the molecular cascade that brings about locomotor sensitization is triggered by events that take place in the ventral tegmental area and the events there, in turn, induce neurobiological adaptations in the nucleus

accumbens that mediate the actual expression of sensitization (Carlezon, Jr. & Nestler, 2002). Interestingly, the trigger process appears to require activation of D1 receptors (Bjijou, Stinus, Le Moal, & Cador, 1996; Carlezon, Jr. & Nestler, 2002; Vezina, 1996), raising the hypothesis that acceleration in the emergence of compulsive behavior observed in the present study, involves a D1 receptor pathway. This hypothesis is consistent with neuroanatomical evidence indicating D1 receptors on dynorphin neurons and terminals (Curran & Watson, Jr., 1995; Gerfen et al., 1990; Le Moine & Bloch, 1995; Le Moine, Normand, & Bloch, 1991), and gains support from our present finding of increases in the proportion of D1-receptors in the high affinity state upon repeated stimulation of kappa opioid receptors (Figure 6) indicating that such indirect stimulation can indeed have an impact on the activity of D1 receptors; moreover, using a similar drug treatment protocol as in the present study, we showed recently that repeated stimulation of kappa opioid receptors elevated in the nucleus accumbens mRNA levels for the D1 receptor (Perreault, Graham, Scattolon, Wang, Szechtman, & Foster, 2007). Thus, distinct brain regions may mediate the development and the expression of compulsive checking though it remains to be determined whether those regions correspond to the ones implicated in locomotor sensitization—the ventral tegmental area and the nucleus accumbens, respectively.

The present study indicates that the contribution of dopamine receptor changes to sensitized responding is not straightforward. Specifically, chronic treatment with U69593 alone, quinpirole alone, or both drugs together, all produced an equivalent increase in the proportion of dopamine receptors in the high affinity state (Figure 6) even though only the latter two treatments induced locomotor sensitization and compulsive checking. Clearly, while $D2^{High}$ elevation appears necessary for psychostimulant-induced sensitization (Seeman et al., 2005; Seeman et al., 2006), it is not a sufficient condition for the expression of sensitized behavior.

Nevertheless, the possibility remains that increases in D2High are more tightly coupled to the development of sensitization, as would be apparent if dopamine receptors were measured during the course of repeated treatment rather than as it was done in the present study, following the last test (injection 15).

The observed differences of kappa agonist co-treatment on development versus expression of sensitized responding suggest the involvement of kappa opioid receptors in acquisition of new behaviors but a less consistent role in the modulation of well-established responses. In this context, the quinpirole OCD model suggests that for long-standing OCD symptoms the therapeutic benefit of kappa drugs is questionable, a suggestion consistent with the limited and mixed benefit of opiates in clinical treatment of OCD (Fontenelle, Nascimento, Mendlowicz, Shavitt, & Versiani, 2007; McDougle, Barr, Goodman, & Price, 1999). By the same token, an opposite but related implication of the observed dichotomy is the possibility that agonists of kappa opioid receptors are potentially pathogenic, especially when combined with dopamine stimulants. This potential liability warrants attention in light of rising recreational use of the psychotropic mint *Salvia divinorum* (Gonzalez, Riba, Bouso, Gomez-Jarabo, & Barbanoj, 2006) that contains as an active ingredient Salvinorin A, an agonist of kappa opioid receptors (Chavkin et al., 2004; Roth et al., 2002; Sheffler & Roth, 2003). Our quinpirole OCD model suggests that in the context of polysubstance abuse involving dopamine hyperactivity, the addition of Salvinorin A may induce symptoms of OCD or other sensitization-related disorders with surprising rapidity.

In all, the present results point to the importance of sensitization mechanisms in the development of quinpirole-induced compulsive checking, mechanisms that are potentiated by costimulation of kappa opioid receptors. Since dynorphin peptides are the endogenous ligands

of kappa opioid receptors (Merg et al., 2006), the quinpirole OCD model suggests that the co-joint activity of the dynorphin opioid system may contribute to the pathogenesis of OCD by accelerating the neurobiological cascade triggered by dopamine hyperactivity. Considering that development and expression of sensitization may involve distinct neural substrates (Carlezon, Jr. & Nestler, 2002), the quinpirole model suggests that a similar distinction may exist for the neurobiology of OCD. Although it is not known what are the functions normally engaged by the co-joint activity of the dopamine and endogenous opioid systems, one may be related to facilitation of survival-related motivated behaviors (Kelley, Baldo, & Pratt, 2005), perhaps by ensuring the rapidity of relevant learning (Petrov, Nizhnikov, Varlinskaya, & Spear, 2006; Petrov, Varlinskaya, & Smotherman, 2000). Conceivably, the normal interaction between dynorphin and dopamine systems may be similarly important in facilitating the activity of the security motivation system, a motivational system whence OCD pathology may emerge (Szechtman & Woody, 2004; Szechtman & Woody, 2006; Woody & Szechtman, 2005).

Figure 1. Effect of U69593 co-treatment (0.3 mg/kg) on the development of locomotor sensitization to repeated injections of quinpirole (0.5 mg/kg, every 3-4 days) in a large open field. Left *y*-axis represents total distance that rats moved during the 55 min session while the right *y*-axis shows the equivalent distance per min. Bars represent group means. Lines represent values of individual rats; line patterns correspond to the ranking of the individual in terms of mean distance across 10 injections. Lines with circles indicate rats portrayed in Figure 2. The parameters of the hyperbolic function (not plotted) that fits mean data of VQ and UQ groups are given in Table 1. Significant differences at each injection are indicated in Table 2. Legend: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Figure 2. Trajectories of locomotion in a 55 min session during injections 1 to 10 shown by 4 rats (top panel) and measures of their performance on injection 10 for compulsive checking (bottom panel, left) and locomotion (bottom panel, right). The selected rats are identified in Figure 1 by a line with circle symbols; rats US17, VQ32 and UQ09 have equivalent mean performance for distance traveled during chronic treatment; rat VS33 has a comparably lower mean performance but it is the highest amount in the VS group. In top panel, the light gray boxes indicate placements in the open field of 4 small objects; the grid of horizontal and vertical lines indicates the virtual demarcation of the open field into 25 locales. Legend: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole..

Figure 3. Effect of U69593 co-treatment during the course of treatment with quinpirole on spatial distribution of locomotion as measured by: (**a.**) path stereotypy index, (**b.**) 2 standard deviational ellipse, (**c.**) density of paths, and, (**d.**) relative focus on key locale.

Values are mean±SEM. The smooth lines fitted to VQ and UQ means in (**a.**) and (**c.**) represent the hyperbolic function with parameters presented in Table 1. Significant differences at each injection are indicated in Table 2. Legend: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Figure 4. Context-dependency of quinpirole-induced compulsive checking as assessed by the object rotation test. The first bar of each pair indicates the mean number of visits on injection 10 when the arrangement of objects in the open field was as usual: an object at a preferred locale (site 14) and no object at a non-preferred place (site 18). The second bar of each pair shows the number of visits on the object rotation test (injection 11) when the spatial arrangement of objects in the open field was rotated by 180 degrees such that an object was no longer present in locale 14 but one was now located at site 18. The numbering of the locales is as described (Szechtman, Sulis, & Eilam, 1998) and refers to the bottom right corner (site 14) and the bottom left corner (site 18) of the open field. The change in the trajectories of locomotion produced by the shift in the spatial arrangement of objects on injection 11 is illustrated by the path plots for rat VQ32 (upper trace) and rat UQ09 (lower trace), which can be compared to their respective trajectories in Figure 2. The gray rectangles indicate the usual position of the objects (before the object rotation test); the position of the objects at injection 11 can be discerned from the concentration of paths at the new location of the objects in the open field (top and bottom left corners, and one square to the right for the two inside objects). * $p<.05$, paired t-test, compared to performance at injection 10. Legend: VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Figure 5. Effect of U69593 co-treatment on development of compulsive checking induced by quinpirole as indexed by (**a.**) absolute and (**b.**) relative frequency of visits to home base, (**c.**) absolute and (**d.**) relative return time to home base, (**e.**) number of locales visited before returning to home base, and (**f.**) duration of visit to home base. Values are mean±SEM. The smooth lines fitted to VQ and UQ means in (a.) and to VQ group in (b.) represent the hyperbolic function with parameters presented in Table 1. Significant differences at each injection are indicated in Table 2. Legend: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Figure 6. Effect of chronic U69593 and quinpirole treatments on dopamine receptors supersensitivity in the nucleus accumbens and the striatum, measured 4 days after the last open field test. Values are mean+SEM. * $p<.05$, compared to VS rats. Legend: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Table 1: Estimates ±SE of parameters of a hyperbolic function fitted to data presented in Figures 1, 3 and 5.

Figure	Variable	Group	Parameter[1]				r^2
			I_{50}	R_{max}	n	R_{min}	
1	Average distance (m/min)	VQ	6.08±1.23	6.65±1.06	2.41±0.56	1.49	0.978
		UQ	4.71±0.35	11.48±0.65*	2.47±0.30	2.33	0.993
3a	Path stereotypy (ratio)	VQ	5.83±1.28	4.60±0.48	3.05±1.17	2.75[2]	0.950
		UQ	2.39±0.19*	5.89±0.13*	3.46±.90	3.13	0.959
3c	Density of paths (% area)	VQ	4.76±0.61	32.59±2.49	3.14±0.94	14.8	0.958
		UQ	3.75±0.16	37.25±0.69	4.80±0.88	19.6	0.985
5a	Frequency of checking (#)	VQ	6.57±1.21	170.6±27.1	2.63±0.58	40.6	0.981
		UQ	3.08±0.35*	193.7±4.99	3.52±0.61	67.5	0.982

[1] Equation (see Methods) fitted to the data of the indicated groups shown in Figures 1, 3 and 5. I_{50} is the number of drug injections required to reach the half-maximal response, R_{max} is the maximal response, n is a parameter describing the sigmoidicity of the curve, R_{min} is the lowest response that served as a fixed parameter in the equation, and r^2 indicates the square of the correlation coefficient between raw and fitted data. Standard error (SE) refers to the standard error of the estimate of the parameter; the estimate of each parameter is statistically significant For groups absent from the table, a significant fit of the function to the data did not exist. Group abbreviations correspond to those in the figures: VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

[2] *Rmin* was fixed close to the value found previously (Dvorkin, Perreault, & Szechtman, 2006) as using the present actual minimum did not yield a reasonable fit.

* $p<.05$ compared to VQ group, *t*-test.

Table 2: Summary of significant differences between groups for data presented in Figures 1, 3 and 5.

Variable	Figure	Statistically significant comparisons (p<.05)[1]
Distance (m)	1	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 2-10; UQ *vs.* VQ: inj 3-10
		VQ *vs.* VS: inj 3,5-10; VQ *vs.* US: inj 6-10
Path stereotypy (ratio)	3a	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 1-10; UQ *vs.* VQ: inj 1-10
		VQ *vs.* VS: inj 5-10; VQ *vs.* US: inj 6-10
2 standard deviational ellipse	3b	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 1-10; UQ *vs.* VQ: inj 2-5
		VQ *vs.* VS: inj 1-10; VQ *vs.* US: inj 1-10
Density of paths (%)	3c	UQ *vs.* VS: inj 2-10; UQ *vs.* US: inj 4-10; UQ *vs.* VQ: inj 4,5,9
		VQ *vs.* VS: inj 3,5-10; VQ *vs.* US: inj 6,9,10
		US *vs.* VS: inj 8
Relative focus on key place	3d	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 1-10; UQ *vs.* VQ: inj 2-5
		VQ *vs.* VS: inj 1,2,5-10; VQ *vs.* US: inj 1,2,4-10
Frequency of checking	5a	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 1-10; UQ *vs.* VQ: inj 2-10
		VQ *vs.* VS: inj 5-10; VQ *vs.* US: inj 5-10
Relative frequency of checking (ratio)	5b	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 2-10; UQ *vs.* VQ: inj 3,4,10
		VQ *vs.* VS: inj 2,3,5-10; VQ *vs.* US: inj 5-10
Recurrence time of checking (s)	5c	UQ *vs.* VS: inj 2-10; UQ *vs.* US: inj 1-10
		VQ *vs.* VS: inj 5-10; VQ *vs.* US: inj 1,3,5-9
Relative recurrence time of checking (ratio)	5d	UQ *vs.* VS: inj 2-9; UQ *vs.* US: inj 2
		VQ *vs.* VS: inj 4-10
Stops before returning to key locale (#)	5e	UQ *vs.* VS: inj 1-10; UQ *vs.* US: inj 1-10; UQ *vs.* VQ: inj 2,3
		VQ *vs.* VS: inj 1,2,5-10; VQ *vs.* US: inj 1,2,4-10
Length of check (s)	5f	UQ *vs.* VS: inj 2-10
		VQ *vs.* VS: inj 2,6-10
		US *vs.* VS: inj 2,6-10

[1] Results of statistical comparisons at each injection for estimated marginal means and 95% confidence intervals, obtained in a Group (4) by Injections (10) repeated measures ANOVA. VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole.

Table 3: Measures of checking behavior on a test for compulsive checking (injection 10), on the object rotation test (injection 11), and on the final test before sacrifice (injection 15). Measures are those established to identify compulsive checking as described in the Methods. Values are mean±SEM.

Variable	Test	Group[1]			
		VS	US	VQ	UQ
Frequency of checking	Inj 10	12.2±1.7	25.6±4.5	138.0±14.9**	197.2±11.6**
	Inj 11	16.0±1.4†	24.7±3.6	136.3±12.3**	170.4±14.2**†
	Inj 15	10.7±2.3	22.4±3.2	177.5±13.5*†	193.0±12.2*
Relative frequency of checking (ratio)	Inj 10	2.9±0.1	3.7±0.3	7.1±0.2**	5.8±0.3**
	Inj 11	3.5±0.1†	3.9±0.2	6.2±0.3**†	5.4±0.4**
	Inj 15	2.6±0.3	3.7±0.4^{+}	6.6±0.3*	5.8±0.2*
Recurrence time of checking (s)	Inj 10	122.2±30.9	77.2±13.0	13.5±1.8*	13.3±1.3*
	Inj 11	105.8±16.6	118.2±16.6†	16.6±1.8*	16.9±2.2*†
	Inj 15	241.9±107.5	112.8±24.8^{+}	12.3±1.1^{+}	12.8±1.5^{+}
Relative recurrence time of checking (ratio)	Inj 10	0.34±0.07	0.25±0.04	0.12±0.01^{+}	0.23±0.02
	Inj 11	0.33±0.04	0.34±0.03†	0.17±0.02*†	0.23±0.01*
	Inj 15	0.60±0.17	0.34±0.06^{+}	0.16±0.01^{+}	0.20±0.02^{+}
Stops before returning to key locale (#)	Inj 10	4.9±0.3	4.9±0.6	2.2±0.1*	2.7±0.2*
	Inj 11	4.8±0.4	4.6±0.3	2.8±0.3*†	3.2±0.4*
	Inj 15	6.8±1.1	4.2±0.3^{+}	2.4±0.1*	2.8±0.2*
Length of check (s)	Inj 10	170.9±46.0	71.3±15.3^{+}	13.1±2.3^{+}	3.6±0.4^{+}
	Inj 11	90.3±28.8	31.2±9.8^{+}†	8.7±1.6^{+}†	4.0±0.7^{+}
	Inj 15	242.5±74.7	83.0±30.2^{+}	7.1±0.8^{+}†	4.4±0.4^{+}

[1] VS = vehicle plus saline, US = U69593 plus saline, VQ = vehicle plus quinpirole, UQ = U69593 plus quinpirole. N=10/group except that only 6 of the rats in the VS group were tested on injection 15.
$^{+}$ *vs.* VS, * *vs.* VS and US, ** *vs.* any other group, Duncan multiple range test ($p<.05$).
† $p<.05$, paired *t*-test, compared to same treatment group on injection 10.

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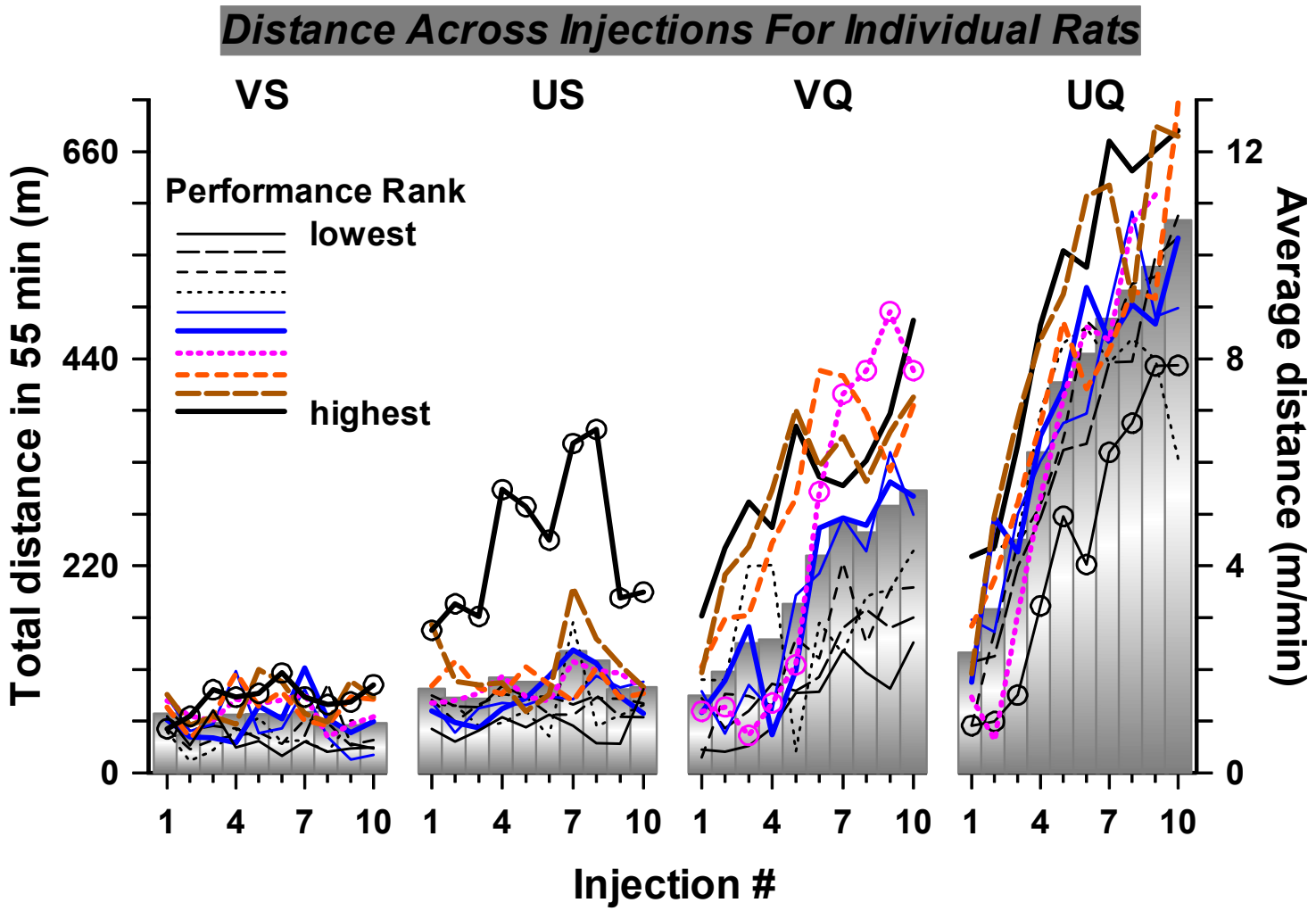
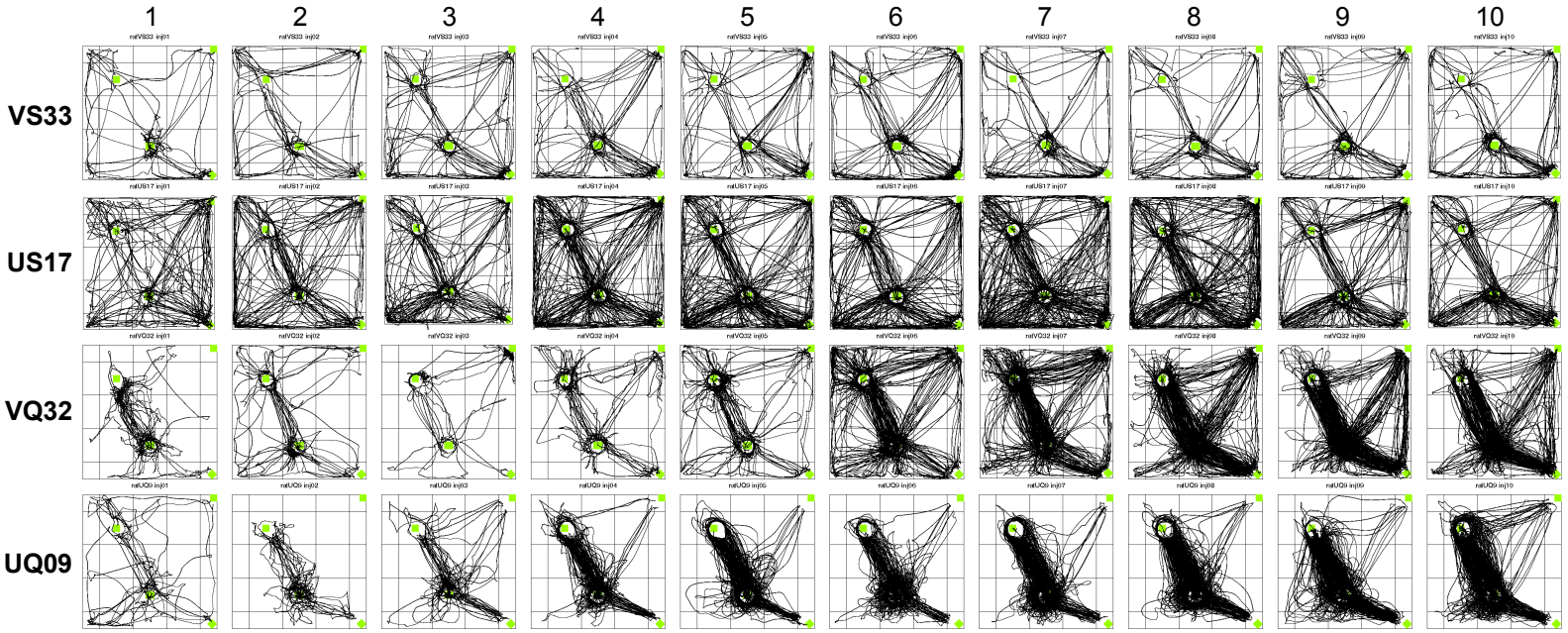
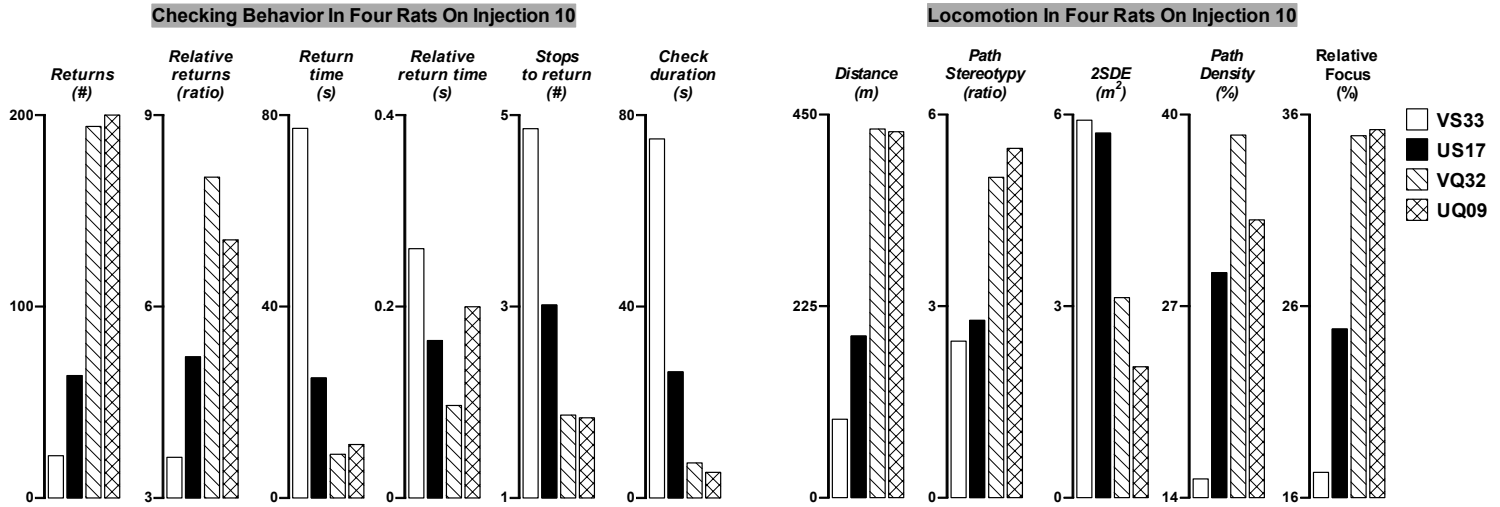
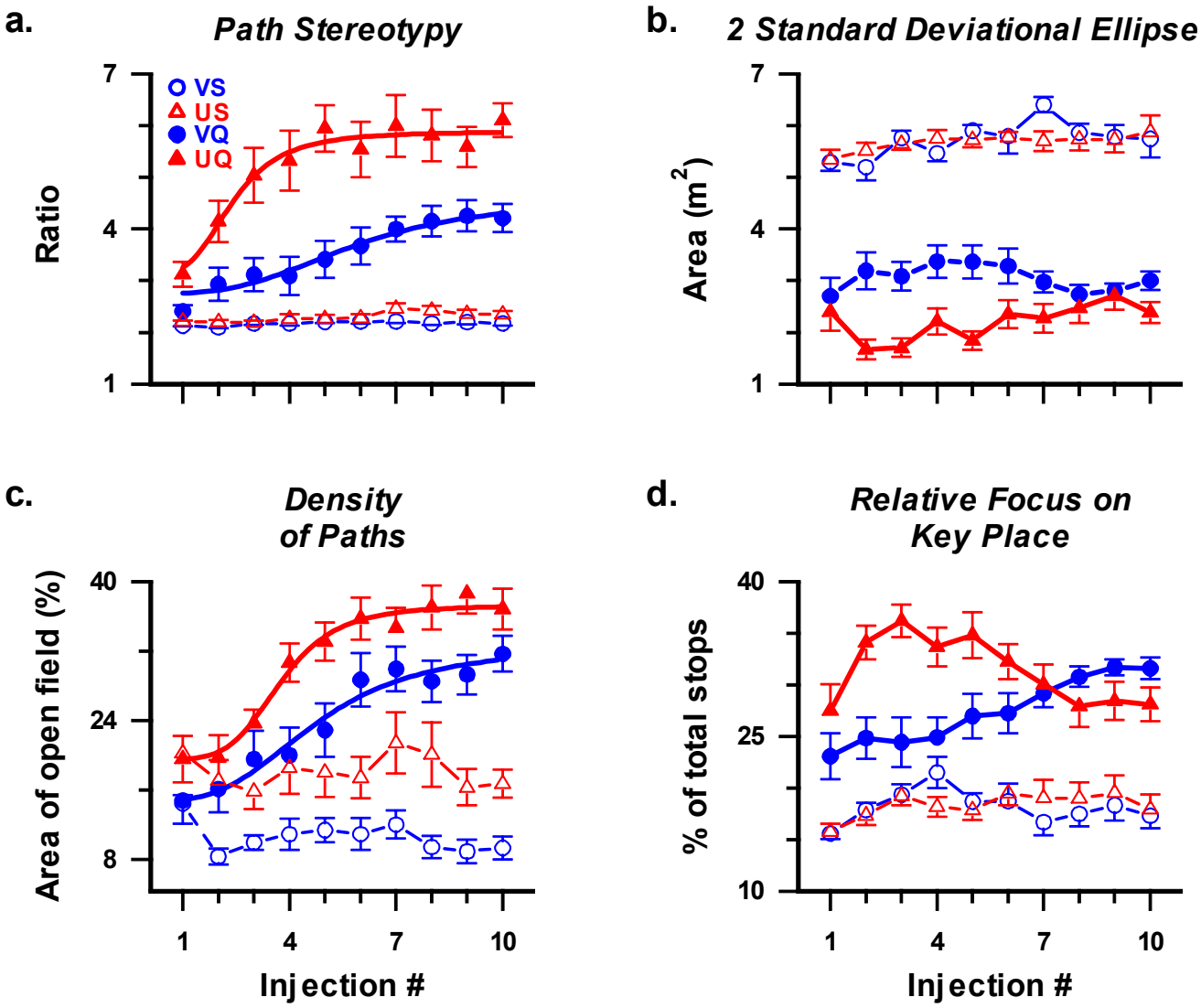


Perreault - Fig. 1

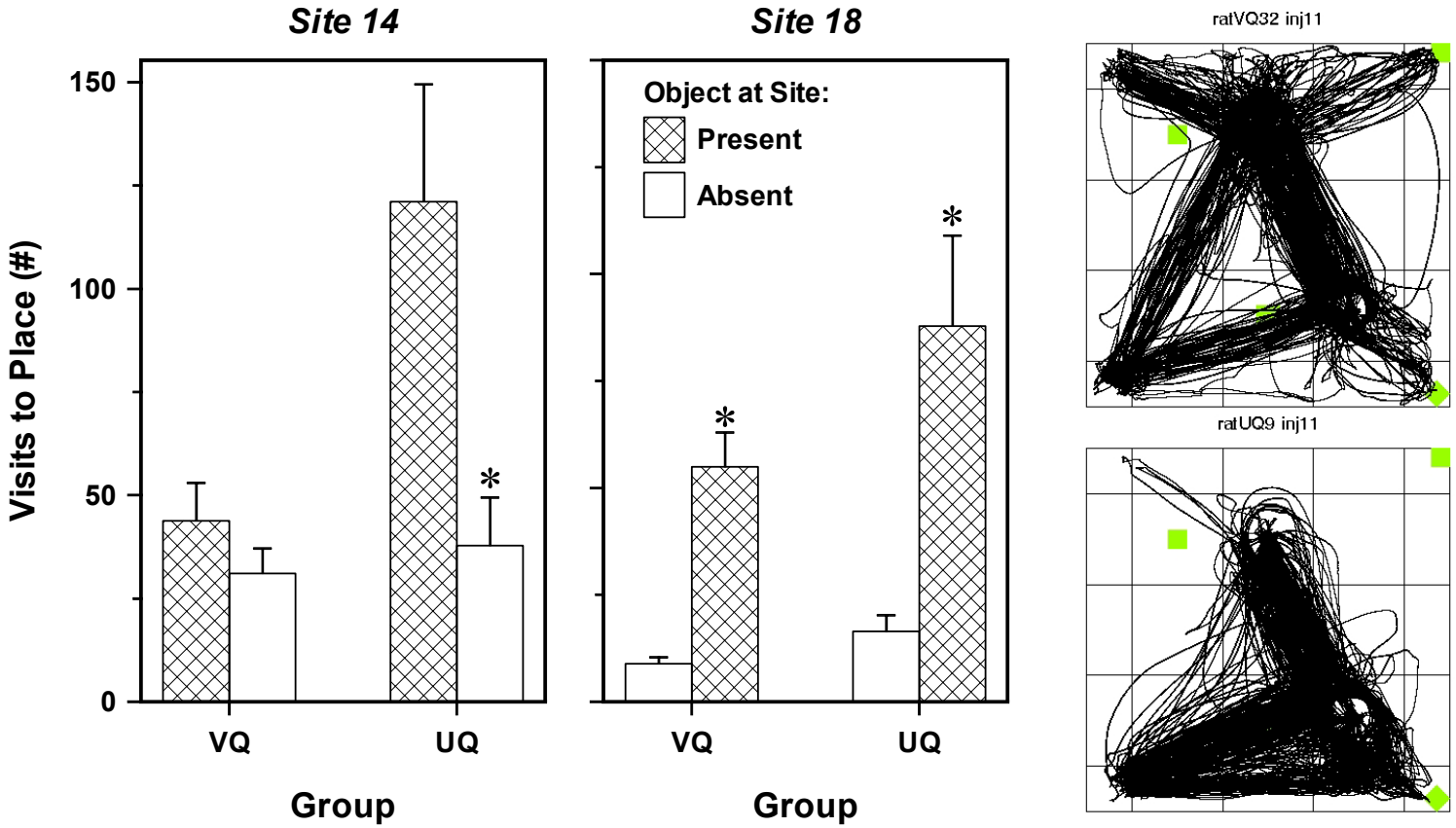




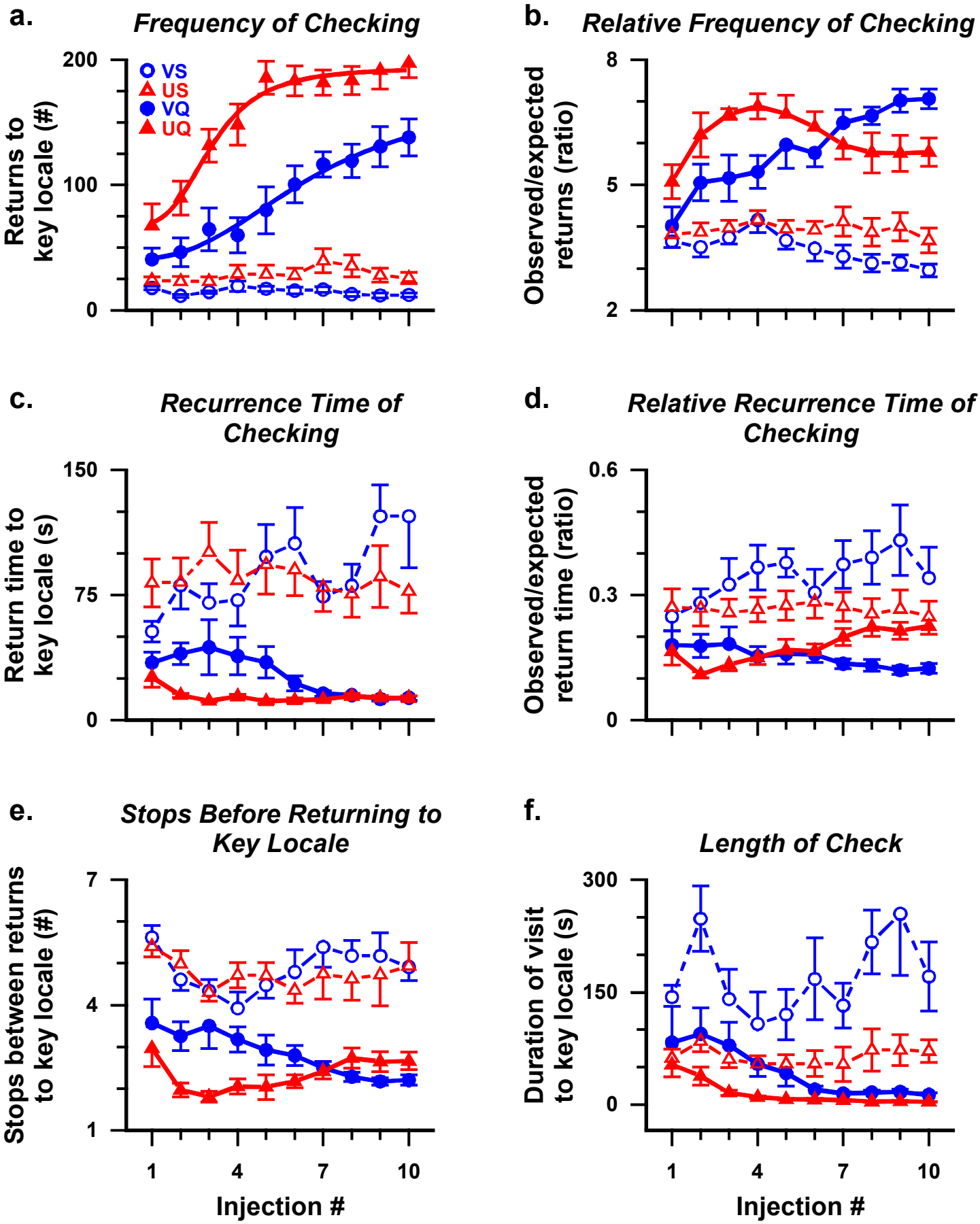
Perreault - Fig. 2



Perreault - Fig. 3



Perreault - Fig. 4


a.
Frequency of Checking
Returns to key locale (#)
200
100
0
VS
US
VQ
UQ
b.
Relative Frequency of Checking
Observed/expected returns (ratio)
8
5
2
c.
Recurrence Time of Checking
Return time to key locale (s)
150
75
0
d.
Relative Recurrence Time of Checking
Observed/expected return time (ratio)
0.6
0.3
0
e.
Stops Before Returning to Key Locale
Stops between returns to key locale (#)
7
4
1
1
4
7
10
Injection #
f.
Length of Check
Duration of visit to key locale (s)
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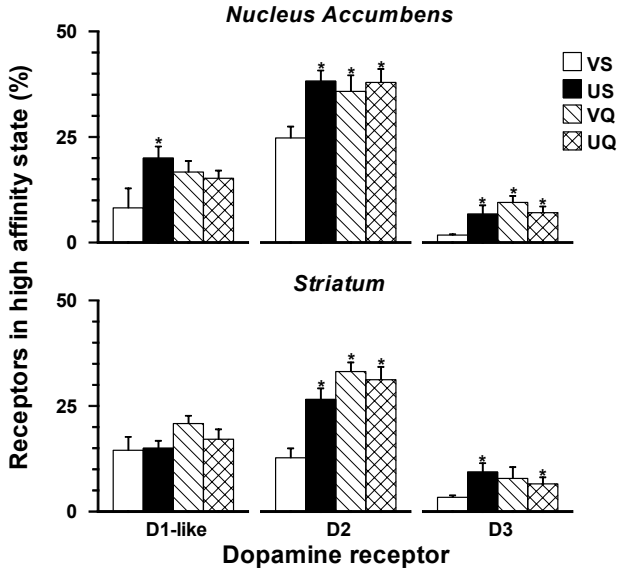
150
0
1
4
7
10
Injection #

Fig. 5



Perreault - Fig. 6