The Morphogenesis of Motor Rituals in Rats Treated Chronically with the Dopamine-Agonist Quinpirole

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Running title: Morphogenesis of motor rituals

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ABSTRACT

In a large open field, rats injected repeatedly with the dopamine agonist quinpirole develop motor rituals that, we show here, evolve through a cascade of four behavioral processes. The first process involves an increase in overall activity. The second process involves an increase in path stereotypy, reflected in traveling repeatedly along the same few paths. The third process is an increase in the frequency of stopping in few places, alongside a decrease in stopping in other places. The fourth process is a decrease in the repetition of movements performed in the specific stopping places. Altogether, these processes culminate in a typical short set of movements comprising a single performance of each movement type. The increased activity leads to a recurrent and stereotyped performance of the entire ritual. Thus, stereotypy does not arise from changes in the content of the behavior patterns used, but in how these patterns are organized temporally and spatially. These results provide a model for the development of motor rituals and their linkage to normal behavior and to the physical properties of the environment.

INTRODUCTION

Motor rituals have been considered as the building blocks of animal behavior, providing the foundation for studying communication, development, neurophysiology, genetics, and evolution (Barlow, 1977). For example, the black-tailed deer (*Odoceileus hemionus*) has a demarcation ritual: it sniffs the ground while punting the soil with one foreleg. Then the deer urinates, while stretching the trunk and bringing the uretra above the sniffed location by stepping with the forelegs but not the hindlegs. Next, forelegs are held rooted while the hindlegs step forward, arching the trunk to bring the anus above the sniffed location and defecating in the same place. This demarcation ritual is repeatedly displayed in fixed locations in the territory (Walther, 1977), illustrating how behavioral templates are implanted in the flow of behavior, and comprise a fundamental part of the behavioral repertoire. Sometimes, the performance of motor templates overrides sensory input, resulting in a disconnection from its function. For example, female gray-lag geese (*Anser anser*) have a typical ritual of rolling eggs back to the nest, placing the bill at the far side of the egg and rolling it to underneath sternum. When the egg is removed after the goose has already started the returning ritual, the goose will complete the ritual despite the lack of the egg (Lorenz & Tinbergen, 1939). When the behavior does not seem to have an obvious goal, and in addition the incidence of a motor ritual increases above a certain frequency, it is defined as a *stereotyped behavior* (Fox, 1965; Hutt & Hutt, 1965; Mason, 1991).

Immelmann & Beer (1989) proposed that ritualization (development of motor ritual) occurs through "*increase in conspicuousness by simplification and "exaggeration" of form, repetition (usually rhythmical), emphasis of particular components, slowing down or speeding up of performance, addition of morphological support such as coloration and stereotypy. In contrast to their unritualized antecedents, ritualized behavior patterns typically show considerable constancy in vigor and rapidity with which they are performed.*" The concept of ritualization was first applied in phylogenetic processes, and then in ontogenetic studies, where it was applied in describing how highly variable behavior gives rise to rigidly stereotyped patterns in adults (Immelmann & Beer, 1989). In order to study the

development of motor rituals, three main approaches may be applicable: 1) following the ontogeny of behavior in newborns or juveniles; 2) manipulating the behavior by environmental changes such as bringing wild animals into captivity; and 3) following consequential behavioral changes after administrating psychoactive drug or neurophysiological treatment. Ontogeny of behavior has been indeed an important tool to study the development of species-specific display behavior (Groothuis, 1993), grooming (Golani & Fentress, 1985), gaits (Eilam, 1997) and exploratory behavior (Eilam & Golani, 1988). However, studies in ontogeny cannot separate the development of behavior from the development of the animal. In other words, not only the behavior, but also the animal under test changes in the course of ontogeny (Bekoff, 1989). Surprisingly, only few studies describe the development of caged stereotypies (e.g, Mason & Turner, 1993) despite the vast attention which cage stereotypies receive in the literature. Drug-induced behavior is therefore a relatively simple method to study the development of repetitive motor routines (Lyon & Robbins, 1975; Geyer, 1982; Eilam & Golani, 1994).

Constancy of form in behavior produced through ritualization, or uniform repetition of motor patterns is termed stereotypy (Immelmann & Beer, 1989), typically arising in wild animals in captivity (Hediger, 1964; Meyer-Holzapfel, 1968; Stevenson, 1983), in farm animals (Fraser & Broom, 1990), and after the administration of psychoactive drugs (Robbins & Shakian, 1981). Cronin & Wiepkema (1984) divided stereotypy into pacing rituals (or locomotor stereotypy) and stationary rituals (or focused stereotypy). In pacing, animals travel along fixed paths. For example, polar bears locomote along the same few paths, with a footfall sequence which is performed accurately on the footprints of the previous traveling on this path (Wechsler, 1991) and amphetamine treated rats travel along a fixed path (Schioring, 1971). In stationary stereotypic rituals, vacuous nest-building behavior when a nest already exists was described in sows (Arey et al., 1991), and chewing, licking, or biting, were described in rats under high doses of amphetamine and apomorphine (Costall & Naylor, 1975). We chose to study the development

of motor rituals in rats in the course of chronic administration of the D2/D3 dopamine receptor agonist quinpirole. Previous studies have shown that after 10 injections of quinpirole, rats have locomotor stereotypy (Szechtman et al., 1994) and repetitive motor rituals in specific places (Szechtman et al., 1998). In the present study, we followed only briefly the development of locomotor stereotypy that we described in our past studies (Eilam & Golani, 1994; Szechtman et al., 1994). Instead, we concentrated in the present study on the development of non-locomotory motor rituals. A profound increase in activity, repetitive performance, and a stereotyped/compulsive form of the behavior of rats after 10 injections of quinpirole (Szechtman et al. 1998) provide an excellent substrate to study the development of motor rituals and stereotypy. Specifically, we followed the changes that behavior underwent in the course of 10 consecutive injections of quinpirole, addressing the following questions: 1) how do motor rituals develop; 2) what is the linkage between the rituals and normal behavior; and 3) how does the environment shape the rituals.

METHODS AND MATERIALS

Subjects

Ten Long-Evans male rats (Charles Rivers, St. Constant, Quebec, Canada) weighting 250-300 g at the start of treatment were used. Rats were housed individually in polyethylene cages (35x30x16 cm) in a temperature-controlled room with a 12-hr light-dark cycle and free access to food and water. The experimenter handled rats for 5 days (2 min each day) before the beginning of treatment. All treatments and testing were administered during the light hours.

Drugs

For five weeks, five rats were injected subcutaneously twice a week with quinpirole hydrochloride (RBI, Natick, MA) dissolved in physiological saline (0.5 mg/ml). Equivalent volumes of saline were used for control injections administered to additional five rats. The

particular drug dosage and injection regimen were chosen because the 0.5 mg/kg dose of quinpirole is representative of the behavioral effects induced by doses of the drug from 0.25 to 2.5 mg/kg, and because the effects of chronic treatment reach a plateau after 8-10 drug injections administered 2 to 8 days apart (Eilam and Szechtman, 1989; Szechtman et al., 1994).

Apparatus

Rats were tested in a large open-field (Walsh & Cummins, 1976) that consisted of a mirrored glass table (160 x 160 and 60 cm high) without walls (Eilam and Golani, 1989) placed at least 70 cm from walls of an air-conditioned experimental room illuminated by fluorescent ceiling lights. The apparatus was subdivided into 25 rectangular places (locales) used to define the location of the animal in the field. Four small Plexiglas boxes (each approximately 8 x 8 x 8 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. Behavior was videotaped continuously on a video-cassette recorder together with a computer-readable time code (Telcom Research, Burlington, Ontario, Canada).

Procedure

Immediately after each injection, rats were gently placed individually into the center of the open field and videotaped for 55 min. The videotapes of the injections 1, 3, 5, 7, 9 and 10 were used to obtain the reported measures of the rats' activity. Fifteen minutes during the peak of activity were analyses in each group. In quinpirole-treated rats this period ranges between 40 to 55 min after drug-injection, and in the saline group between 0 to 15 min. The design of the study focused on the development of quinpirole-induced stereotyped behavior across injections.

Behavioral Analysis

A computer interfaced with the video recorder was used to score behavior during playback of the video records, with a resolution of 1/30 of a second, and custom-made software provided

measures of distribution of activity, as described previously (Eilam et al., 1991; Eilam et al., 1992). As noted previously (Eilam and Golani, 1989), in an open field a rat can be either locomoting or not. Periods of *locomotion* comprise continuous forward progression abided between two successive periods of no locomotion or *stops*. During stops rats perform various acts including lateral movements, vertical movements, and grooming. During locomotion such movements are not performed, and the trajectory between two consecutive stops is the *path* of locomotion. Our previous studies revealed that between consecutive stops, rats typically progress in a straight trajectory, and therefore, a score of the sequence of stops adequately represents the paths and the spatio-temporal organization of locomotor behavior. When the path is curved or circular, a score of few additional points (generally one) suffice to describe the path (Eilam & Golani, 1989; Eilam et al., 1989). The following parameters were scored in the present study:

I. Locomotor behavior

Distance traveled: overall distance that a rat traveled during the 15-min observation.

Locomoting time: overall duration of locomoting periods, during which rats accumulate the traveled distance.

Speed: The ratio between the distance traveled and locomoting time. It should be noted that this measure reflects the average speed of actual locomotion and not the mean speed of travel over the entire observation period.

Number of stops: overall number of non-locomoting periods. Stops were assigned to one of 25 predefined zones (‘places’) in the open field.

Stops and time at the home base: home base was defined as the place where a rat spent the longest cumulative duration of non-locomoting periods. These data reflect the number of stops and their total duration.

Stops and time at the second base: the second base was defined as the second in rank of places, in terms of cumulative duration of stopping and number of stops.

Number of excursions: an excursion was defined as round trip to either bases, including traveling between the home base and the second base.

Stops per excursion: number of stops taken between two successive stops at either of the bases.

Total paths, different paths and stereotypy: 'Total paths' is the cumulative sum of paths regardless of their concrete trajectory. 'Different paths' is the different concrete trajectories, and the ratio between the total and different paths is 'path stereotypy', which is the number of repetitive passes along the same path.

II. Behavior during stops

Direction of arrival and departure: Each place was conceived as if it was located in the origin of eight vectors spaced at 45° intervals (example of this system of reference is given in the relevant results). These directions (vectors) were used to represent directions of arrival and departure to the place under analysis.

Lateral movements: Lateral movements of the head only, and of the entire trunk including the head, were scored by indicating their amplitude and the direction to which they were executed (clockwise or counterclockwise). The amplitude of lateral movements was measured by assessing the angular change in the longitudinal axis of the head or the trunk in units of 45º. Thus a score of –1 for the head represented a 45º angular counterclockwise shift of the longitudinal axis of the head, and a score of 2 for the trunk represented a 90º angular clockwise shift of the entire trunk.

Vertical movements: Five types of vertical movements were scored: 1) lowering the head over the edge of the open field; 2) raising the head upwards; 3) rearing on the hindquarters (releasing contact of both forelegs); 4) climbing with one or both forelegs on top of a box; and 5) climbing and standing with all four legs on top of

a box. The last two categories were applicable only in places in which boxes were located.

Grooming and box poking: grooming with the forelegs (Golani & Fentress, 1985) was scored only as the incidence of continuous grooming bouts. Three of the boxes had an opening on one side, into which the rats could insert their head. This activity was scored as 'head poking'.

It should be noted that the separation to horizontal and vertical movements was based on previous studies that demonstrated morphological (Eilam & Golani, 1988) and functional (Grobety & Schenk, 1992) separation of these domains.

Statistics

Analysis of variance (ANOVA) with repeated measures was used to test three differences: 1) between saline and quinpirole groups; 2) across injections in each group; and 3) interaction of group and injections. Significant interaction implies that the behavioral change across the repeated injections of quinpirole differed from that induced by repeated saline injections. Since data on the behavior during stops deviated significantly from normal distribution (Kolmogorov-Smirnov test for normality), analysis was carried out on logarithmatically or square-root-transformed data. For the directions of arrival and departure, a *logit transformation* of the proportions was applied to minimize the effect of the absolute number of stops. Then, a *paired t-test* was used to compare each two directions. The alpha level was set to 0.05 (two-tailed) in all experiments.

RESULTS

In an open field, a rat can be either locomoting or not locomoting (stopping). During locomotion, the rat may only progress in the environment and its behavior may be described by the trajectories of its locomotion. During stops the rat can execute activities such as grooming, rearing on the hindquarters, lateral movements, etc. The following analysis of open field behavior was thus divided into the analysis of 'locomotor activity' and to 'movements during stops'. As explained in the introduction, in the present work the analysis of locomotion was a brief follow up of previous studies, and the focus was on movements during stops. Additionally, the effect of the physical structure of the environment was inspected.

Locomotor activity: Increase in the incidence of stopping at a few specific locations

Activity: Table 1 summarizes changes in activity that took place across injections. As shown, the traveled distance gradually increased in quinpirole-treated rats but was steady in saline-injected rats. Locomoting time was higher in quinpirole-injected rats compared to saline-injected rats, but in both groups it did not change across injections, and speed overlapped in both groups, without not changes across injections. These results indicate that the higher level of locomotion in the quinpirole-treated rats was due to a longer locomoting time, during which they traveled longer distance, yet they locomoted at the same speed of the saline group.

Path Stereotypy: Across injections, quinpirole-treated rats developed repetitive locomotion along the same few paths. To demonstrate this, we defined 'path stereotypy' as the total number of paths that a rat passed, divided by the number of different paths passed by that rat (a path was the trajectory of locomotion between two consecutive stops). As shown in Table 1, under quinpirole, path stereotypy increased across injections compared to saline group. Path stereotypy increased in the quinpirole-treated group since the number of total paths increased while the number of different paths did not. In the saline group, path stereotypy, the number of total paths, and the number of different paths were relatively steady and low across injections. Therefore, quinpirole

treated rats traveled longer distances, spent more time locomoting, and took more stereotypic paths compared to saline treated rats.

Table 1

Stopping Places: Table 2 shows four changes in stopping that occurred across injections in the quinpirole, but not the saline group: 1) the total number of stops increased; 2) the number of stopping places decreased; 3) the number of places to which the rats paid 50% and 80% of their stops decreased; and 4) the number of stops in the home base and the second base profoundly increased. Therefore, compared to saline treated rats, quinpirole treated rats stopped more frequently in the bases and in few additional places.

Table 2

Across injections, the number of stops per excursion between the home base and the second base, or in roundtrips to each of these places, was relatively steady in the saline group. Yet the number of stops per excursion declined to less than 1 in the quinpirole-treated group (Figure 1), since these rats typically traveled from base to base either directly or with one stop in between bases.

Figure 1

<u>Movements during stops: Emergence of fixed motor rituals at the stopping places</u>

In order to uncover the development of motor rituals in specific places, the behavior was analyzed across injections in three open-field locales (places): 1) corner with a box at the front of the open field; 2) corner without a box at the front edge of the open field; and 3) locale near the center of the open field with a box in it. These places were selected for detailed analysis because of the high frequency of stops that the rats paid to them, and their physical properties that allow distinguishing the role of these features in shaping the behavior. The three places were amongst the five most visited places in each of quinpirole-treated rats, and among the eight most visited places in the saline-treated rats.

Stop duration: In the three stopping places which were selected for analysis, there was a decline in the duration of stops across injections of quinpirole compared to a slight increase in the saline group (Figure 2). As shown below, the diminish in the duration of stops in quinpirole rats was associated with a concomitant decrease in the number of lateral and vertical movements.

Figure 2

Behavior in the front corner with a box:

Arrival and departure: This corner had three possible directions of arrival to it and departure from it (along the edges, and in between them). Starting at injection 3 of quinpirole, one of the arrival directions gradually dominated, culminating in 80% of the arrivals in injection 10. Under saline, the incidence of arriving in the three directions overlapped throughout. However, both groups have a preferred direction of departure from the place. Nevertheless, this preference was more constant in the quinpiorle group, compared to its fluctuations in the saline groups. Figure 3 illustrates the development of the preference to a specific direction of arrival and departure in an exemplary saline-treated rat (a) and an exemplary quinpirole-treated rat (b).

Figure 3

Changes in the movements that the rats executed during stopping in this place are detailed in Table 3. In this table, each row stands for a certain movement type, e.g. – the first row describes the incidence of clockwise lateral head movements, while the fifth row describes the amplitudes of clockwise lateral head movements. The overall trend of change in each movement type is depicted in the column "Trend", followed by the mean±SEM measured in each injection. Following is a brief summary of these changes.

Lateral movements: Both the incidence and the amplitude of head movements diminished under quinpirole but increased or remained unchanged under saline. In contrast, while the incidence of trunk movements was very small, their amplitude increased. Thus, quinpirole rats executed less

lateral movements per stop, yet the fewer lateral trunk movements were carried out in large amplitudes. In the saline group, the incidence of lateral movements typically increased, while their amplitude did not change and was less than half of the average amplitude of the quinpirole group.

Vertical movements: As in the lateral domain, quinpirole-treated rats executed less vertical movements, compared to saline-treated rats. On average, saline rats executed one or more vertical movements, whereas the quinpirole rats executed less than one vertical movement per stop.

Grooming and head poking into the box: Under quinpirole, grooming was eliminated entirely from the behavioral repertoire, and head poking was rare. In contrast, these behaviors were frequent in the saline group.

Table 3

Movement sequencing: Quinpirole treated rats arrived to this corner in a preferred direction, stopped there for a brief period and executed few movements, and then departed from the corner by a preferred direction. Moreover, during stops in this corner, movements were executed in a typical sequence as shown in Figure 4. Three changes that were shown above to occur across injections of quinpirole, but not under saline, are illustrated in this figure: 1) decrease in the types of movements which establish the behavioral repertoire; 2) decrease in the overall number of movements which were executed in a stop (regardless of their type); and 3) decrease in the number of repetition of each movement type. As a result of these processes, there was a decline in the variability and richness of the behavioral repertoire during stopping in this corner. The sequence of movements was not entirely predictable, yet, at injection 10 quinpirole rats had a relatively fixed ritual in this corner: they arrived in a preferred direction, established snout contact with the box, made a vertical movement followed by a wide clockwise lateral movement, and departed from the corner by a preferred direction. A vertical movement could precede the

lateral movement, and a repetition of any of these movements was relatively infrequent. In all, therefore, the qunipirole-treated rats repeatedly visited this corner, and gradually developed there a ritual that was based on fewer movements and repetitions, compared to saline-treated rats. This raised the question whether these behavioral processes were specific to this place or whether they reflect a more general change that would also underline the behavior in other places.

Figure 4

Behavior in front corner without a box:

Arrival and departure: Like the previous corner, this corner also has three possible directions of arrival and departure. Both the quinpirole and the saline groups had the same preferred direction of arrival (data not shown). In departure they also had a preferred direction of departure, but this was different in the two groups (data not shown).

The changes in the movements that the rats executed during their stops in this place are detailed in Table 4. This table design is similar to Table 3. Following is a brief summary of these changes.

Lateral movements: The incidence of head movements was consistently less than 1 per stop under quinpirole, but not saline. The amplitudes of these head movements were typically similar. The incidence of lateral trunk movements was lower under quinpirole. However, a large counterclockwise lateral movement of the trunk characterized the behavior during stops in this corner. Rats in the saline group typically display more than one lateral movement of the head and of the trunk, both in the clockwise and the counterclockwise directions.

Vertical movements: As in the lateral domain, quinpirole-treated rats executed less vertical movements, compared to saline-treated rats. In average, saline rats executed one or more vertical movements, whereas the quinpirole rats executed less than one vertical movement per stop.

Table 4

Movement sequencing:

Figure 5 presents sequences of the movements during visits to this corner. As revealed in these movement sequences, two changes occurred in the behavior of quinpirole, but not saline treated rats in this corner: 1) decrease in the overall number of movements which were executed in a stop (regardless of their type); and 2) decrease in the number of repetition of each movement type. The decrease in the number of repetitions was most obvious in the vertical movements, which by injection 10 reached less than a third of their incidence on injection 1. A third process, a decrease in the types of movements was observed in the corner with the box (Figure 4) but not in this corner. Indeed, most sequences comprised the same types of movement, and there was no indication for a relatively fixed order in performing these movements. Table 5 summarizes the decreases in repetition of each movement type and in the overall number of movements, as revealed in Figure 5.

Figure 5 and Table 5

In all, both at the corner with a box and the corner without a box, the order of movements was not predictable. Yet, quinpirole-treated rats arrived to and departed from the corner by a preferred direction; moreover, they stopped at the corner for a short period of time during which they executed a short sequence of movements. While in the corner with the box rats turned clockwise before departure, in the corner without the box they turned counterclockwise before departure.

Behavior in a place with a box near the center of the open field:

Arrival and departure: Unlike corners in which arrival and departure could take only three directions, arriving and departing a place in the center of the open field was possible in 8 predefined directions. Nevertheless, quinpirole treated rats had one preferred direction of arrival and departure, which progressively dominated across injections (data not shown). In saline-

treated rats, the incidence of arriving and departing in the different directions overlapped (data not shown).

The changes in the movements that the rats executed during their stops in this place are detailed in Table 6, which has the same design of tables 3 and 4. Following is a brief summary of these changes.

Lateral movements: The trends in this place were similar to that shown above for the corners: the incidence of head movements was typically less than 1 per stop in quinpirole rats, and trunk movements, especially counterclockwise movements, were large in amplitude.

Vertical movements: As shown for the corners, quinpirole-treated rats executed less vertical movements, compared to saline-treated rats.

Grooming and head poking into the box: Under quinpirole, grooming was eliminated entirely from the behavioral repertoire, and poking the head into the box was rare. In contrast, these behaviors were frequent in the saline group.

Table 6

Movement sequencing:

Figure 6 presents sequences of the movements during visits to this place. These sequences were sampled in the same procedure of figures 4 and 5. As revealed in these movement sequences, quinpirole but not saline treated rats executed fewer movements and fewer repetitions in this corner, but this lower incidence remained relatively constant across injections and did not decline further, as found for the places described previously above. In both the saline and the quinpirole groups there was a flexible order of movements. Indeed, most sequences comprised the same types of movement, and there was no indication for a relatively fixed order in performing these movements. Therefore, the behavior of the quinpirole-treated rats in this place with box near the center of the open field but away from the front, shared several features with the behavior at the front corners with/without boxes: 1) the rats arrived and left the place in a

preferred direction, and this preference increased across injections; 2) the incidence of lateral and vertical movements decreased; 3) the duration of stopping decreased; and 4) grooming was not executed.

Figure 6

Effect of the physical structure of the environment

The above analysis of the behavior indicated that physical characteristics of stopping locations might influence the spatio-temporal structure of behavior at these locations. Indeed, the rigidity of the sequences of movements (Figures 4,5,6) was high in front corner with box, lower in front corner without box, and even lower in a box near the center. To further illustrate this influence, six places (the above three and additional three places) with different physical characteristics were examined. As indicative of the preference to a place we used the cumulative number of stops. In Table 7, the six places are ranked according to the cumulative number of stops across injections. As shown in Table 7, the order of the ranked places (left hand column) was almost identical in both the quinpirole and saline groups. This indicates that the impact of the physical environmental characteristics was similar in both groups. Inspection of the table reveals hierarchical three factors that account for the preference to the different places: 1) proximity of the place to the front edge of the open field; 2) presence of box in the place; and 3) location of the place in the center or in a corner of the open field.

Table 7

DISCUSSION

Motor rituals develop in the course of chronic administration of quinpirole through four parallel processes: 1) increase in activity; 2) repetitive locomotion along the same few paths; 3) increase in the incidence of stopping in a few specific places with a decrease in stopping at other places; and 4) emergence of relatively fixed motor rituals in stopping places. Therefore, stereotypy does not arise from changes in the content of the behavior patterns, but from changes in the spatial and temporal organization of normal behaviors. In the following discussion, we consider the role of motor rituals as the building blocks of stereotyped behavior, the gradual transition from normal to quinpirole-induced stereotypy, and the coupling between behavior and physical structure of the environment.

Motor rituals – the building blocks of stereotypies

During stops, normal rats execute a sequence of lateral and vertical movements together with typical activities like grooming and rearing. These movements are executed and repeated in an apparently random order, resulting in the flexible and relatively unpredictable normal behavior as seen in the present study in saline treated rats. In the quinpirole-injected rats, however, motor rituals emerged across repetitive injections. Two processes characterized the establishment of the rituals: 1) within a stop, repetition of movement types declined to a single performance of each type of movement; 2) across repetitive stops in a specific place, movements were executed in the same direction and with the same amplitude. This processes occurred non-monotonically across injections, culminating in the rat arriving in a specific direction, executing a short sequence of movements, and leaving the place by a specific fixed direction. The ritual ultimately consisted of a "skeleton" of single lateral movement that was executed in a fixed direction and amplitude and single vertical movement. The increase in the frequency of stops and the short sequence of non-repetitive movements yield form constancy, which is characteristic of behavior produced through

ritualization (Immelmann & Beer, 1978). This constancy, together with a parallel increase in passing the same routes ("locomotor rituals"), produces ‘drug stereotypy’. Thus, motor rituals, which are executed during stops, are the building blocks of stereotyped behavior.

The behavior of quinpirole rats is apparently stereotypic, i.e., repetitive, seems purposeless, and carried out in relatively fixed form. 'Stereotypy' is generally associated with increase in repetition. However, the present results demonstrate that a decrease in the repetition of movements is a cardinal process in the establishment of a stereotypic pattern, producing short sequences with low variability, as shown in Figures 4,5,6. It is therefore required to define what is the unit of repetition in stereotypy. In the present results, a stop is the repetitive behavioral unit, or ritual, that is comprised of movements that are the building blocks. In ritualization, the number of building blocks decreases and ultimately each block appears only once in a stop. Moreover, across injections each type of movement (= building block) takes similar direction and amplitude, consolidating short and unvarying rituals that are performed repeatedly, resulting in form constancy. Robbins (1977) proposed that behavior is stereotypic when intuitively and subjectively we can fix the limits of the repetitive bout of behavior. The present results provide simple means for the definition of the repetitive bout: separating the behavior to locomoting and non-locomoting periods, analyzing the trajectories of locomotion as a sequence of stops, and analyzing the sequence of movements during stops. This dichotomy seems very appropriate since stereotypies are divided to pacing and stationary (Stevenson, 1983). In captive wild animals, pacing takes the form of a linear, circular or figure 8-shaped trajectory (see Hediger, 1964 for review). Under drugs, pacing, or locomotor stereotypy, is generally viewed as a set of consecutive linear trajectories between few stopping places (Schioring 1971; 1979; Geyer, 1982; Eilam & Golani, 1994; Szechtman et al., 1994; Paulus & Geyer, 1997). In stationary (also termed ‘focused stereotypies’ in drug-induced behavior), the animal displays a set of repetitive movements while it is stationary and does not locomote. This dichotomy of behavior to

locomoting and non-locomoting periods was utilized in the analysis of normal and drug-induced behavior in previous studies (e.g., Eilam & Golani, 1989; Eilam et al., 1989; Szechtman et al., 1994; 1998) and in the present work.

The emergence of stereotyped rituals under quinpirole occurred in different intensities at different locations, resulting in a spectrum that ranges from more flexible and normal-like behavior in one place, up to the very rigid, stereotypic-like behavior in another place. However, this variability may be specific to quinpirole stereotypy since under amphetamine, the deviation from normal behavior occurs simultaneously in all places (Eilam & Golani, 1990). Nonetheless, the continuous changes across injections of quinpirole make it hard to draw the borderline between normal and stereotyped behavior. Such a border is often arbitrary, and may not exist at all when stereotypies become ingrained in the normal flow of behavior (Mason, 1993). For this reason, the isolation of the motor ritual as the elementary unit of stereotypy is crucial for understanding the behavior.

Motor rituals in normal and in stereotyped behavior

Mason (1991) drew three similarities between stereotyped and normal behaviors: 1) stereotypy is rigid and resistant to changes, like many normal behaviors such as grooming or display; 2) stereotypy is purposeless, but so do many normal acts such as ‘habits’; and 3) like normal behavior, once stereotypy develops, it becomes detached from the stimuli that evoked it and may appear in entirely different situations. In all, stereotyped behavior is typically a normal behavior that turns to be repetitive and established while loosing flexibility (Mason, 1991). For example, stereotypy in mice was shown to develop from normal activities like jumping or chewing, although it then detached from these behavioral sources (Wurbel et al., 1996). In the same vein, the present results emphasize that normal and stereotyped behaviors differ in structure, not in content. This is apparent in the description of the establishment of relatively stable motor rituals that are not detached from normal behavior, neither different in building

blocks: the same components that comprise normal behavior also comprise the stereotyped performance seen in injection 10, but in stereotypy the components are structured into short and repetitive rituals compared to the relatively long and flexible sequences of movements in normal behavior.

Increase in activity as a prerequisite for the emergence of repetitive behavior

The present results reveal that locomotor stereotypy evolves through repetition of the same few paths, and not from a change in the number of paths. Indeed, drug studies showed that increase in activity beyond a certain margin results in stereotypy (e.g., Eilam et al., 1989; Muller et al., 1989). Eilam et al. (1991) proposed that there are two antagonistic mechanisms that keep the animal organized in time and space: the first leads to locomotion in a confined space and is activated when there is an increase in activity; the second leads to expansion of locomotion and is activated when activity decreases. According to this model, the increase in activity under chronic injections of quinpirole lead to stereotyped locomotion in a confined area whereas the lower activity under saline lead to diverse locomotion expanding over larger area. Indeed, previous studies have suggested that increase in activity is a prerequisite in the establishment of repetitive and less variable motor performance (Mason 1993).

The increased level of activity was represented by the increase in the traveled distance that by injection 10 was 2.5 fold higher than in injection 1, and an increase in speed which by injection 10 was twice than that calculated in injection 1. The duration that the rat spent locomoting (locomoting time) was higher than in saline rats, but steady across quinpirole treatment. This process is apparently different from another study on the establishment of locomotor stereotypy under quinpirole (Szechtman et al., 1994) where stereotypy evolved through an increase in speed followed by an increase in the time spent locomoting. As a result the traveled distance increased, and locomotor stereotypy emerged (Szechtman et al., 1994). The

difference between these studies seems to stem from different sampling methods: in the present study behavior was described only at the of peak activity, between 40 and 55 min after injection. During this period, quinpirole rats were active and locomoting throughout. In the previous study, behavior was measured in seven 3-min intervals that were sampled over two hours after drug administration. This period includes initial immobility followed by gradual increase in activity (Eilam & Szechtman 1989; Szechtman et al., 1994). However, regardless of whether the profound increase in activity under quinpirole involves an increase in speed and locomoting time or only an increase in speed, the increase in activity is the keystone in the establishment of motor rituals.

The emergence of motor rituals in rats treated chronically with quinpirole corroborates previous model on the role of activity, time, and space in the establishment of stereotypies and motor rituals (Serruya & Eilam, 1996): changing one or more of these variables induces repetitive motor "stereotyped" rituals. Under drugs, time and space are held constant, but activity is increased and the rate of repetition of activity increases at specific places or routes (Geyer 1982; Muller et al. 1989; Eilam & Golani 1989; 1990; Eilam et al. 1991; Szechtman et al. 1994). In caged animals, the space available for moving shrinks compared to the natural home range. Thus the normal level of activity has now to be executed within a confined environment, resulting in stereotypy that is a typical response of animals to restricted environment (Morris, 1964), and is particularly severe in animals that naturally inhabit a large home range or territory. When space and activity are maintained at normal levels, behavior seems "normal" and not "stereotyped". However, if observation time is extended, an accumulation of activity in a specific natural space (i.e., "place" or "location") is revealed as repetitive motor routines (Serruya & Eilam, 1996). In all, therefore, stereotyped behavior is derived from normal behavior under physiological or physical change, which result in exaggerated repetition of specific behavioral units in specific time and place.

How does stereotypy develop?

Lyon & Robbins (1975) proposed that stereotypy develops through increased activity that prevents the animals from completion of movements, curtailing single movements or sequences of movements. According to this model, when activity increases, the duration of each movement is shortened until it is not completed and terminated by the beginning of another movement. Further increase in activity results in shortening the sequence of movements by eliminating movements. These processes were illustrated in the behavior of rats that were trained to avoid electric shock by bar pressing, but when treated with drugs that induced hyperactivity, these rats shorten their behavior to just touch the bar without pressing it enough to prevent the shock. Another illustration was that of stereotyped rats that chewed food without swallowing and digesting it.

The present analysis of the establishment of stereotypic motor rituals during stops revealed increased activity, but no curtailing or exclusion of movements, except for grooming which was eliminated even under acute injection (Eilam & Szechtman, 1989; Eilam et al., 1989). In contrast to the model of Lyon & Robbins, the present results show increase in amplitude of specific movements (e.g. - lateral movements to a preferred direction), and almost identical motor repertoire in quinpirole and in saline rats. Similarly, in an account on the development of route stereotypy (Eilam & Golani, 1994), it was argued that stereotyped routes do not necessarily become shorter, but sometimes even become longer. The discrepancies between the above descriptions and the model of Lyon & Robbins (1975) on the development of stereotypy probably stem from differences in isolating the repetitive behavioral unit in stereotypy. Lyon & Robbins's model could appropriately explain that stops became frequent and shorter, but would fail to describe the processes that occur within a stop – the establishment of motor rituals.

The role of the physical structure of the environment in shaping the behavior

The present results reiterate previous studies on the role of corners and objects in shaping open field behavior (Eilam & Golani, 1989; Szechtman et al., 1993; 1998), adding a hierarchy of three environmental cues that were involved in shaping the behavior under quinpirole. In terms of activity, proximity to the front of the open field was the leading environmental factor converging activity to the front of the open field, as shown previously in quinpirole-treated rats (Szechtman et al., 1994; 1998). Second was the presence of an object (box), and third was the corner. These factors affect not only the distribution of activity in the open field, but also the movements that comprised the motor ritual. This is illustrated by the preferred direction of the lateral movement of the trunk that precedes the departure from the different places. As summarized in the following diagram (Figure 7), these movements, which were described in results, lead in the shortest way to the front, overriding an alternative preference or bias to execute lateral movements in specific direction (clockwise or counterclockwise). The hierarchy of the environmental factors was the same in quinpirole and saline rats, suggesting that the role of these factors in shaping the behavior was identical, but the higher level of activity under quinpirole emphasized the role and hierarchy of these factors. The same factors seem to affect, sometimes in different way, the behavior under amphetamine. Indeed, Amphetamine treated rats tend to visit more frequently the corners, but move away from the front of the open field (Eilam & Golani, 1990).

Figure 7

Summary

The present study highlights the role of motor ritual as the building block that constructs stereotypy and describes their emergence across injections of quinpirole, thus extending the study of ritualization from the phylogenetic and ontogentic connection to the development of drug-induced rituals. As typical to the process of ritualization, the motor display in the quinpirole

rats is carried out in a typical intensity and with constancy in form (Immelmann & Beer, 1989). In the quinpirole-treated rats the rituals are equivalent to stops, and the entire behavior is regarded as a set of rituals (stops) in particular places, carried repeatedly and in a typical order. This structure of behavior is reminiscent of our recent observations on compulsive rituals in humans suffering from Obsessive-Compulsive Disorder (OCD). We find that the behavior of these patients is comprised of a set of rituals. Almost every act, as simple as hand washing, dish washing, eating, mouth cleaning, or door opening, is performed in a constant form and intensity, resulting in an endless set of rituals that severely obstruct normal functioning (Zohar, Amiaz, Baram, Szechtman & Eilam; work in progress). This similarity in structure supports our proposal that rituals of quinpirole-treated rats are a plausible animal model of OCD (Szechtman et al, 1998; 2000).

ACKNOWLEDGEMENTS

We thank Ms. G. Shefer and Ms. M. Gurevitz for their help in data acquisition. This study was supported by the Canadian Institutes of Health Research operating grant MT-12852 (HS) and by the Israel Science Foundation, Grant 589-99 to D.E. Henry Szechtman is a Senior Research Fellow of the Ontario Mental Health Foundation.

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TABLES

Table 1: Locomotion: Differences across injections in quinpirole- and saline- administered rats

		1	3	5	7	9	10	Significance
Traveled	Qnp	47.1±17.4	69.2±20	87±17.7	108.2±19.2	108.2±21.5	119.1±23	A: $F_{1,8}$=24; p<0.005*****
Distance (m.)	Sal	8.9±3.9	3.7±1.8	9.2±3.5	9.8±5	12.3±2.5	8±4	B: $F_{1,8}$=5.1; p<0.005
Locomoting	Qnp	139.4±43.2	152.8±35.4	173.4±40.6	195.9±38.8	180.8±34.2	183.6± 33.2	A: $F_{1,8}$=23; p<0.005
Time (sec.)	Sal	15±6.2	7.5±4	17.2±7.47	17.8±10	22.8±4.7	14.6±8	B: ns
Speed	Qnp	0.29±0.05	0.43±0.07	0.55±0.11	0.56±0.03	0.58±0.04	0.64±0.03	A: ns
(m/sec)	Sal	0.35±0.15	0.34±0.15	0.55±0.18	0.39±0.17	0.55±0.03	0.65±0.09	B: $F_{1,8}$=2.7; p<0.05
Total	Qnp	47.2±19.5	94.4±30.6	117.6±30.4	138.6±32.6	135.8±30.1	136.6±30.4	A: $F_{1,8}$=9.6; p<0.05*****
Paths	Sal	85.2±11.7	33.4±8.9	37.6±11.2	31±8.5	36.6±11.3	37.4±13	B: ns
Different	Qnp	23.8±8.2	29±9	30.8±6.8	24.6±4.8	25±3.2	25±3.7	A: ns
Paths	Sal	44.2±5.5	21.8±4.7	20.6±5.1	19±3.5	20.4±4.9	19.4±5.2	B: $F_{1,8}$=2.8; p<0.05*****
Path	Qnp	1.9±0.2	3.2±0.4	3.8±0.4	5.6±0.7	5.2±0.9	5.3±0.5	A: $F_{1,8}$=78; p<0.001*****
Stereotypy	Sal	1.9±0.1	1.5±0.1	1.8±0.1	1.6±0.2	1.6±0.2	1.7±0.2	B: $F_{1,8}$=7.6; p<0.001

A: is assigned for p values for the effect between the groups of saline and quinpirole.

B: is assigned for p values for the effect within the groups (across injections).

* indicates an additional significant interaction group x injections ($p<0.05$).

Table 2: Locomotor behavior: Differences across injections in quinpirole- and saline-administered rats

	Quinpirole injection						Saline injection					
	1	3	5	7	9	10	1	3	5	7	9	10
Total number of stops	37	77.8	90.6	110	104.4	115.2	71.4	29.2	34.4	32	33.4	35
Total number of stopping places	12	13	12	10.4	10.2	10.2	15.8	11.4	10.8	11.6	10.6	10.4
Number of stopping places – 50%	3	1.9	1.7	1.6	1.6	1.5	4.9	4.5	4.5	4.9	4.7	4.7
Number of stopping places – 80%	9	5.8	4.9	4.8	4.8	4.5	10.7	9.7	9.8	9.8	9.5	9.8
Number of stops at the home base	10.6	27.8	29.2	41	37.8	43.8	12.4	5.6	6.2	5.2	4.4	5.2
Number of stops at the second base	3.6	13.6	23.4	29.4	25.8	35	7	3	3.2	2.8	3	2.9

Table 3: The *incidence* (in italic format) and the **amplitudes** (in bold format) in front corner with a box.

			Trend	INJ # 1	INJ # 3	INJ # 5	INJ # 7	INJ # 9	INJ # 10	
LATERAL MOVEMENTS										
Head	*Clockwise*	*QNP*	⇩	*0.42±0.31*	*0.23±0.11*	*0.08±0.03*	*0.08±0.05*	*0.04±0.03*	*0.01±0.01*	
		SAL	—	*0.70±0.34*	*2.72±1.10*	*1.32±0.59*	*1.92±0.70*	*2.78±1.62*	*1.85±1.01*	
	Counter-	*QNP*	⇩	*0.49±0.11*	*0.26±0.10*	*0.14±0.02*	*0.15±0.06*	*0.03±0.01*	*0.02±0.01*	
	Clockwise	*SAL*	⇧	*0.59±0.27*	*2.20±0.92*	*1.19±0.56*	*1.38±0.69*	*3.60±2.88*	*3.46±2.92*	
	Clockwise	**QNP**	⇩	**35±20**	**50±15**	**55±15**	**30±15**	**20±0**	**10±10**	
		SAL	—	**40±10**	**40±10**	**40±10**	**45±10**	**50±5**	**50±0**	
	Counter-	**QNP**	⇩	**80±10**	**90±20**	**50±5**	**55±5**	**35±15**	**25±20**	
	Clockwise	**SAL**	—	**50±15**	**65±10**	**70±10**	**90±10**	**35±15**	**30±10**	
Trunk	*Clockwise*	*QNP*	—	*2.42±0.62*	*1.82±0.40*	*1.21±0.08*	*1.27±0.05*	*1.12±0.06*	*1.25±0.05*	
		SAL	⇧	*3.13±0.94*	*4.24±1.53*	*3.47±1.08*	*4.68±1.45*	*7.52±4.87*	*6.10±2.58*	
	Counter-	*QNP*	⇩	*1.44±0.34*	*0.58±0.34*	*0.54±0.08*	*0.29±0.07*	*0.26±0.05*	*0.24±0.05*	*P<0.01*
	Clockwise	*SAL*	⇧	*1.47±0.23*	*3.42±0.75*	*2.12±0.41*	*1.82±0.18*	*3.98±1.80*	*4.18±1.76*	
	Clockwise	**QNP**	⇧	**75±10**	**105±15**	**145±5**	**150±10**	**180±20**	**155±5**	**P<0.001***
		SAL	—	**75±5**	**75±5**	**65±5**	**65±15**	**70±10**	**70±10**	
	Counter-	**QNP**	—	**110±20**	**125±25**	**120±15**	**115±15**	**130±10**	**100±15**	**P<0.001**
	Clockwise	**SAL**	—	**70±5**	**70±10**	**65±5**	**75±5**	**55±5**	**65±5**	
VERTICAL MOVEMENTS										
Head dipping over		*QNP*	—	*0.11±0.08*	*0.37±0.11*	*0.23±0.11*	*0.12±0.06*	*0.13±0.04*	*0.21±0.10*	
The edge		*SAL*	⇧	*1.38±0.30*	*2.21±0.58*	*1.62±0.25*	*1.81±0.51*	*3.12±0.94*	*3.00±1.02*	
Head & Chest up		*QNP*	—	*0.11±0.08*	*1.20±0.41*	*0.19±0.06*	*0.35±0.15*	*0.20±0.07*	*0.20±0.12*	*P<0.05*
		SAL	—	*0.32±0.21*	*2.24±0.90*	*1.79±0.73*	*1.53±0.26*	*2.28±1.71*	*3.31±2.67*	
Climbing on top		*QNP*	—	*0.80±0.42*	*1.33±0.34*	*0.73±0.07*	*0.62±0.15*	*0.68±0.07*	*0.74±0.21*	
of the box		*SAL*	⇧	*1.90±0.42*	*2.45±0.50*	*2.37±0.52*	*2.34±0.91*	*2.87±1.30*	*3.28±1.92*	
GROOMING										
		QNP	—	*0*	*0*	*0*	*0*	*0*	*0*	
		SAL	⇧	*0.20±0.12*	*0.93±0.14*	*0.91±0.41*	*0.90±0.22*	*1.82±1.55*	*4.09±3.26*	
INSERTING THE HEAD INTO THE BOX										
		QNP	—	*0.51±0.21*	*0.63±0.35*	*0.19±0.06*	*0.12±0.04*	*0.04±0.02*	*0.05±0.03*	*P<0.001*
		SAL	⇧	*1.0±0.17*	*2.65±0.61*	*2.26±0.98*	*2.03±0.46*	*3.23±1.54*	*4.1±2.2*	

p values refer to the effect between the groups of saline and quinpirole.

* additional positive interaction ($p<0.001$) which reflects the increased amplitude in the quinpirole compared to the saline group

Table 4: The *incidence* (in italic format) and the **amplitudes** (in bold format) in front corner without a box.

			Trend	INJ # 1	INJ # 3	INJ # 5	INJ # 7	INJ # 9	INJ # 10	
LATERAL MOVEMENTS										
Head	*Clockwise*	*QNP*	—	*0.44±0.23*	*0.19±0.09*	*0.06±0.05*	*0*	*0*	*0.05±0.05*	
		SAL	—	*0.59±0.17*	*1.22±0.34*	*1.55±0.58*	*4.28±3.44*	*1.15±0.47*	*1.08±0.24*	
	Counter-	*QNP*	—	*0.25±0.25*	*0*	*0.45±0.28*	*0.01±0.01*	*0.02±0.01*	*0.18±0.09*	
	Clockwise	*SAL*	—	*0.21±0.09*	*1.1±0.24*	*1.1±0.41*	*3.07±2.24*	*1.1±0.28*	*0.93±0.1*	
	Clockwise	**QNP**	—	**60±15**	**45±0**	**45±0**	**0**	**0**	**45±0**	
		SAL	—	**45±0**	**50±5**	**50±5**	**65±1**	**70±10**	**50±5**	
	Counter-	**QNP**	—	**45±0**	**0**	**45±0**	**0**	**135±90**	**55±10**	
	Clockwise	**SAL**	—	**35±10**	**55±10**	**60±5**	**60±5**	**55±5**	**55±0**	
Trunk	*Clockwise*	*QNP*	⇩	*2.25±0.98*	*0.89±0.54*	*0.47±0.16*	*0.34±0.02*	*0.28±0.15*	*0.16±0.07*	
		SAL	—	*0.88±0.63*	*1.3±0.7*	*0.29±0.38*	*2.13±1*	*1.96±0.84*	*1.22±0.37*	
	Counter-	*QNP*	—	*1.25±0.16*	*1.8±0.82*	*0.97±0.27*	*0.85±0.13*	*1.01±0.1*	*0.79±0.12*	
	Clockwise	*SAL*	—	*1.52±0.25*	*1.42±0.57*	*0.24±0.2*	*2.43±1.66*	*2.58±1.15*	*2.39±0.92*	
	Clockwise	**QNP**	—	**115±15**	**155±35**	**165±30**	**90±15**	**105±20**	**140±45**	
		SAL	—	**80±5**	**100±30**	**155±60**	**85±15**	**100±15**	**105±10**	
	Counter-	**QNP**	⇧	**100±35**	**140±20**	**130±10**	**175±15**	**165±10**	**200±25**	
	Clockwise	**SAL**	—	**95±5**	**80±20**	**95±15**	**100±10**	**80±10**	**70±10**	
VERTICAL MOVEMENTS										
Head dipping over		*QNP*	⇩	*0.94±0.41*	*2.23±1.72*	*0.36±0.10*	*0.36±0.15*	*0.53±0.18*	*0.40±0.10*	
The edge		*SAL*	—	*1.47±0.24*	*2.36±0.77*	*1.42±0.23*	*3.17±2.22*	*2.81±0.69*	*2.12±0.49*	
Head & Chest up		*QNP*	⇩	*1.93±1.55*	*0.78±0.33*	*0.69±0.17*	*0.15±0.07*	*0.29±0.08*	*0.47±0.27*	
		SAL	—	*0.57±0.23*	*1.38±0.48*	*1.90±0.53*	*1.36±0.18*	*2.18±0.59*	*1.08±0.24*	
GROOMING										
		QNP	—	*0*	*0*	*0*	*0*	*0*	*0*	
		SAL	⇧	*0.2±02*	*1.05±0.09*	*0.93±0.25*	*0.91±0.09*	*1.81±1.21*	*4.02±3.08*	
HEAD POKING: N/A (no box in this corner)										

Table 5: Repetition of movements across the injections presented in Figure 5. Note the decrease in the incidence of each movement type and in the total number of movements, compared to the constancy in the saline groups.

Type of Movement	Mean number of repetition					
	Quinpirole			Saline		
	Injection 1	Injection 5	Injection 10	Injection 1	Injection 5	Injection 10
A	1	1	1	1	1	1
D	2.8	2	1.4	4.7	4.7	4.5
E	3.8	1.3	1.1	2.8	2.1	2.8
H	0.8	1	1	0.8	0.8	0.8
Total per stop	**8.4**	**5.4**	**4.5**	**9.2**	**8.6**	**9.1**

Table 6: The *incidence* (in italic format) and the **amplitudes** (in bold format) in a stopping place far from the front with a box.

			Trend	INJ # 1	INJ # 3	INJ # 5	INJ # 7	INJ # 9	INJ # 10	
LATERAL MOVEMENTS										
Head	*Clockwise*	*QNP*	—	*1.17±0.07*	*1*	*2.00±0*	*1.75±0.16*	*0*	*1±0*	P<0.01 *
		SAL	—	*1.20±.0.09*	*1.50*	*1.60±0.19*	*1.25±0.11*	*1.59±0.04*	*1.58±0.16*	
	Counter-clockwise	*QNP*	—	*1.19±0.08*	*1.33±0.26*	*2±0.63*	*1±0*	*2*	*1.17±0.13*	P<0.01
		SAL	—	*1.22±0.1*	*1.13±0.13*	*0.98±0.25*	*1.26±0.09*	*2.18±0.56*	*1.56±0.16*	
	Clockwise	**QNP**	—	**52±4**	**45±0**	**90±0**	**79±8**	**0**	**45±0**	
		SAL	⇧	**54±5**	**34±24**	**72±9**	**56±7**	**71±2**	**71±8**	
	Counter-Clockwise	**QNP**	—	**54±4**	**60±13**	**90±32**	**45±0**	**90±0**	**52±7**	
		SAL	⇧	**55±5**	**51±7**	**44±13**	**57±5**	**98±28**	**70±8**	
Trunk	*Clockwise*	*QNP*	—	*2.0±0.07*	*2.72±0.42*	*3.14±0.34*	*2.72±0.17*	*2.92±0.3*	*2.29±0.34*	
		SAL	—	*1.61±0.2*	*1.85±0.4*	*2.09±0.65*	*1.62±0.17*	*1.58±0.23*	*1.49±0.26*	
	Counter-clockwise	*QNP*	—	*2.08±0.11*	*2.38±0.46*	*3.06±0.37*	*3.79±0.44*	*2.44±0.55*	*3.33±0.36*	P<0.005
		SAL	—	*1.7±0.07*	*1.25±0.15*	*1.59±0.17*	*1.85±0.27*	*1.8±0.19*	*1.52±0.09*	
	Clockwise	**QNP**	—	**90±4**	**122±21**	**141±17**	**123±9**	**131±15**	**103±17**	
		SAL	—	**72±10**	**83±20**	**94±33**	**73±9**	**71±12**	**67±13**	
	Counter-clockwise	**QNP**	⇧	**94±5**	**107±23**	**138±18**	**170±22**	**110±28**	**150±18**	
		SAL	—	**76±4**	**56±8**	**71±8**	**83±14**	**81±10**	**68±4**	
VERTICAL MOVEMENTS										
Head dipping over edge				N/A (no edge in this place)						
Head & Chest up		QNP	⇩	0.46±0.18	0.39±0.19	0.26±0.19	0.22±0.14	0.17±0.14	0.08±0.08	
		SAL	—	0.42±.08	0.4±0.11	0.43±0.24	0.8±0.33	0.98±0.23	0.61±0.36	
Climbing on top of the box		QNP	⇩	2.24±1.55	0.34±0.11	0.55±0.17	0.43±0.17	0.27±0.12	0.78±0.17	
		SAL	—	0.1±0.07	0.1±0.1	0.23±0.15	0	0.38±0.19	0.04±0.03	
GROOMING										
		QNP	—	0	0	0	0	0	0	
		SAL	—	0	0	0.03±0.02	0.13±0.1	0.05±0.05	0.04±0.01	
HEAD POKING										
		QNP	—	0.21±0.1	0.1±0.1	0.23±0.2	0.14±0.06	0	0.05±0.04	
		SAL	—	0.24±0.11	0.33±0.12	0.32±0.09	0.57±0.15	0.68±0.2	0.16±0.1	

p values refer to the effect between the groups of saline and quinpirole.

* additional positive interaction ($p<0.001$) which reflects the increased amplitude in the quinpirole compared to the saline group

Table 7: Changes in the number of stops in six places with different physical properties

QUINPIROLE GROUP

Place	Physical Characteristics				Injections Number					
	Front	Box	Corner	Center	1	3	5	7	9	10
4	+	+	-	+	10.6±4.5	27.8±5.9	29±3.7	40.8±7.1	37.8±5.7	43.2±7.7
30	+	+	+	-	3.2±1.3	14±3	22.8±5.4	29.2±5.8	26.6±6.2	36±9.7
50	+	-	+	-	2±0.8	3±0.7	6.4±2.1	10.8±2.9	11.8±3.3	8±1.8
7	+	+	-	+	5.8±1.7	6.8±3.5	10.2±6.3	5.6±0.7	4±0.9	8.6±2.9
10	-	+	+	-	4±1.3	3.4±1.1	2±0.6	1.2±0.3	1.4±0.9	1.4±0.5
70	-	-	+	-	1.4±0.6	0.8±0.3	0.4±0.2	0	0.2±0.2	0.2±0.2

SALINE GROUP

Place	Physical Characteristics				Number of injections					
	Front	Box	Corner	Center	1	3	5	7	9	10
4	-	+	-	+	16±1.2	8.4±1.5	9.2±1.5	7.4±1.5	6.4±1.3	7.2±1.4
30	+	+	+	-	8.4±1.2	3.8±0.5	4.2±0.7	4±0.9	3.8±1	4.4±1.1
7	+	+	-	+	9.6±0.9	3.4±0.6	4±1.3	3.6±0.8	2.4±0.8	4±1.1
50	+	-	+	-	3.6±0.8	2.2±0.7	3.4±1.2	2.6±0.5	3.4±1	4±1.1
10	-	+	+	-	1±0.4	0.2±0.1	1±0.4	0.8±0.3	1.2±0.1	1±0.4
70	-	-	+	-	0.4±0.3	0	0.4±0.2	1.2±0.5	1±0.2	0.6±0.2

Figure captions

Figure 1: Mean+SEM of the number of stops per excursion. An excursion is a trip between the two bases or a round trip to one of the two bases. The number of stops per excursion thus indicates in how many additional places the rat stopped before returning to one of the bases. As shown, in quinpirole rats ( ) typically the number of stops per excursion was lower than 1, implying that they typically traveled directly or with one stop between bases. In contrast, saline rats ( ) typically traveled with more than one additional stop between bases.

Figure 2: Stop duration (sec.) in the three locales selected for analysis: A. front corner with a box; B. front corner without box; and C. box near the center of the open field. In the three places, the duration of stops diminished to minimum under quinpirole ( ), but typically increased under saline ( ).

Figure 3: The establishment of preference to certain directions of arrival and departure in an exemplary saline-injected rat (A) and an exemplary quinpirole-injected rat (B). Each incidence of arrival is represented by a dark circle ( ) and each incidence of departure by a light circle (O), both depicted along the corresponding direction of arrival/departure (along the edges of the open field or diagonally). N values provide the number of stops in this corner. As shown, there is no directional preference across injections of saline. In contrast, across injections of quinpirole, the rat developed a preference to arrive at the corner from a diagonal direction and a preference to depart from the corner along the bottom or diagonal directions.

Figure 4: Movement sequences during stops in front corner with box across injections 1,5,and 10 in saline (left) and quinpirole (right) treated rats. The temporal order of the movements is

aligned from left to right along the rows, and letters were assigned to movements as follows: A- arrival in the preferred direction; B – snout contact with the box; C – climbing on the box; D – lateral movement; E – vertical movement; F – grooming; G – head poking; H – departure in the preferred direction.

Using the procedure of sampling that is detailed below, the figure presents for each injection up to 15 sample sequences (up to 3 sequences/rat). Each row (sample) represents the sequence of movements preformed by a rat during one visit; the identity of the rat is mentioned on the left of each injection. Thus the sequences present the overall effect of each injection in the quinpirole and in the saline-injected groups.

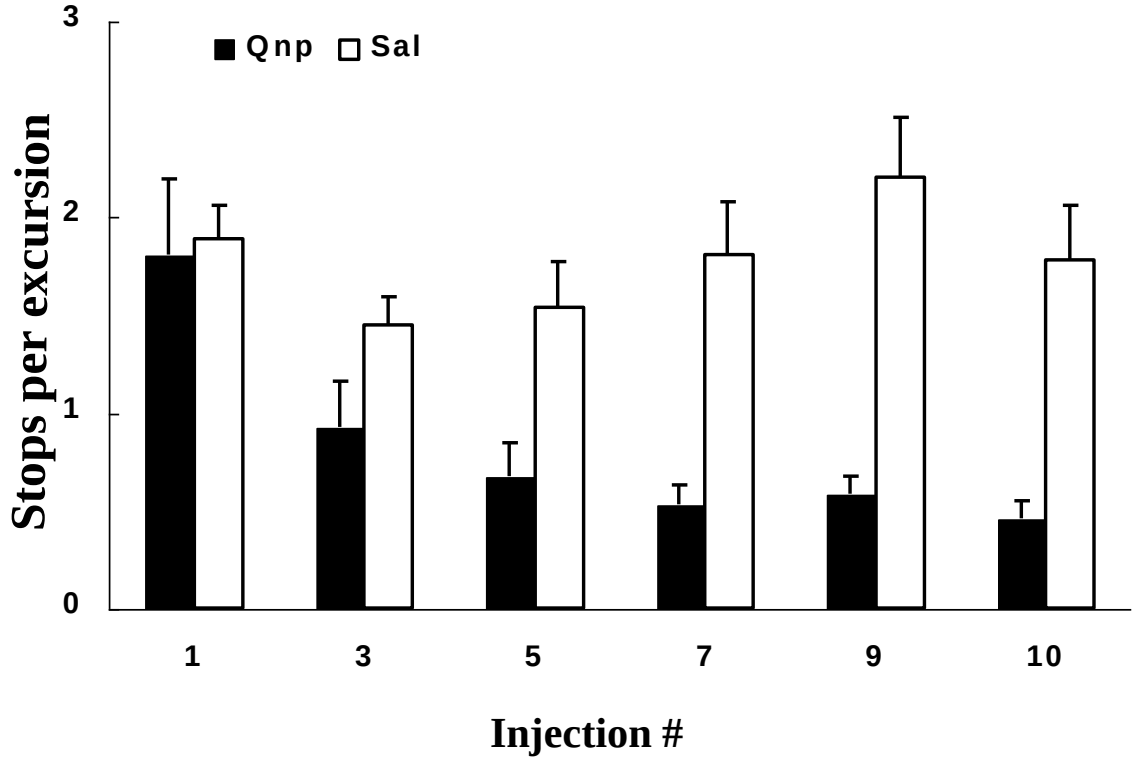
Sampling of sequences: For each rat, three sequences were sampled: 1) the sequence of movements during the first stop in this location during the scored interval; 2) the sequence of movements during a stop in the middle of the scored interval; and 3) the sequence of movements during the last stop in this place during the scored interval (see Methods section for details on these intervals). However, not all rats stopped in that location in the specified intervals, and some of the saline animals skipped the location throughout. In these cases the number of sequences for a rat was less than 3. For instance, injection 1 of the saline group provides 2 sequences of rat 1, 3 sequences of rats 2, 3 and 4, and 0 sequences for rat 5. Injection 1 of quinpirole comprises 3 sequences for each of the rats 1,2,3,4,and 5.

Figure 5: Sequences of movement during stops in front corner without box across injections 1, 5, and 10 in saline (left) and quinpirole (right) treated rats. The structure of the figure and the procedure of sampling the sequences are similar to those used in Figure 4.

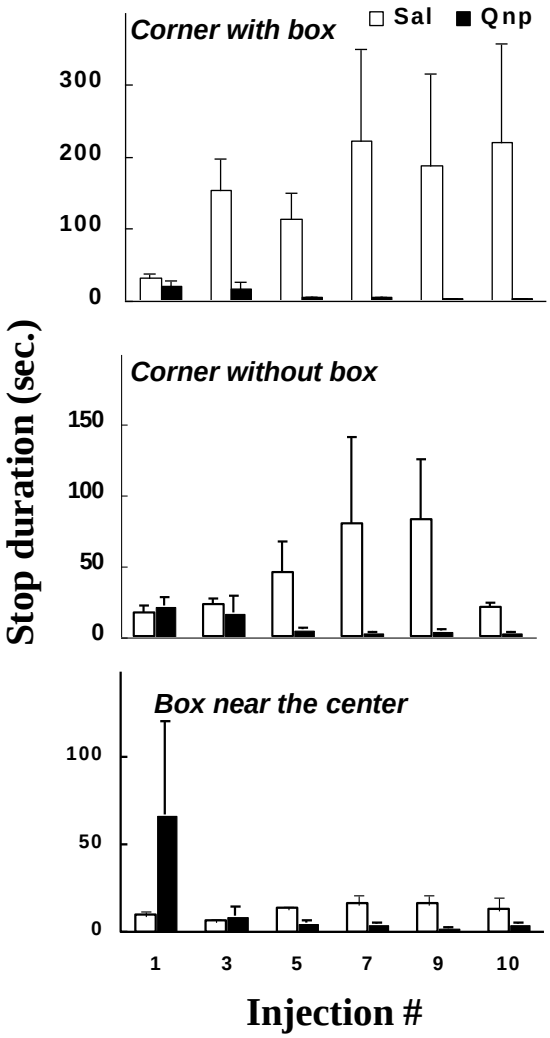
Figure 6: Sequences of movement during stops at a box near the center of the open field across injections 1, 5, and 10 in saline (left) and quinpirole (right) treated rats. The structure of the figure and the procedure of sampling the sequences are similar to those used in Figure 4.

Figure 7: Turning to leave the place in the shortest way to the front. A sketch of the open field and the 4 boxes are presented in black. The frequently traveled paths between the objects are depicted in light gray, and the directions of the lateral movement of the trunk that precedes the departure from the different places are depicted in the rounded arrows. These lateral movements lead in the shortest way to the front (bottom edge).

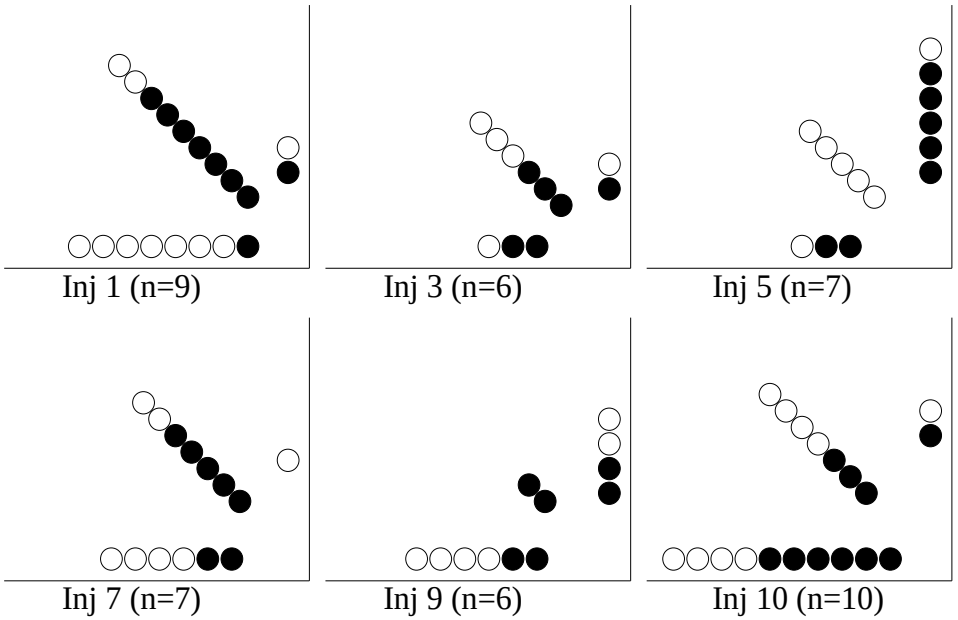
Eilam:Figure 1



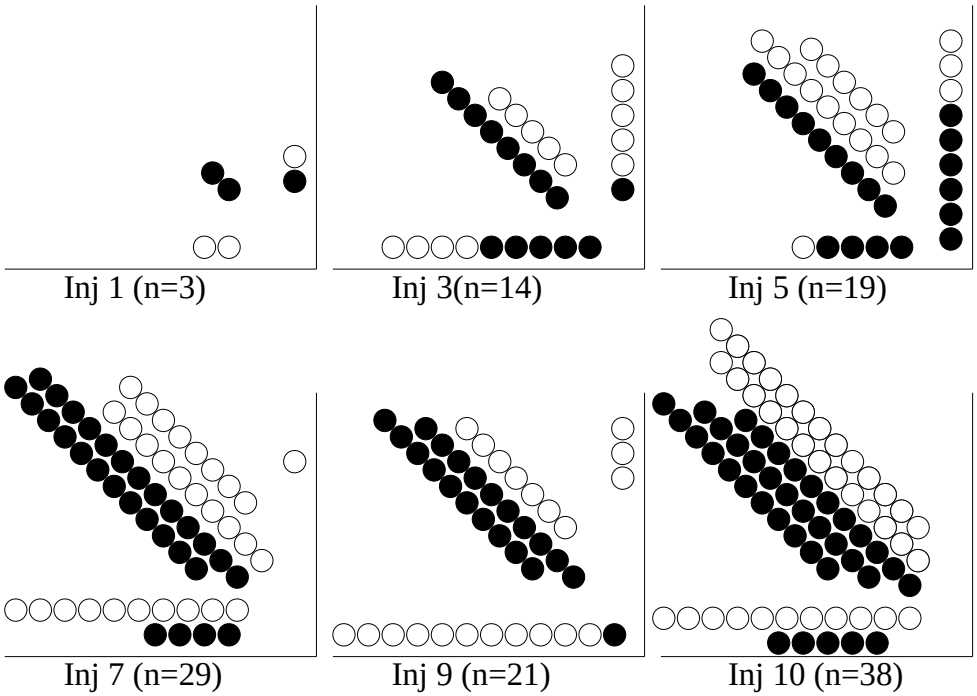
Eilam: Figure 2



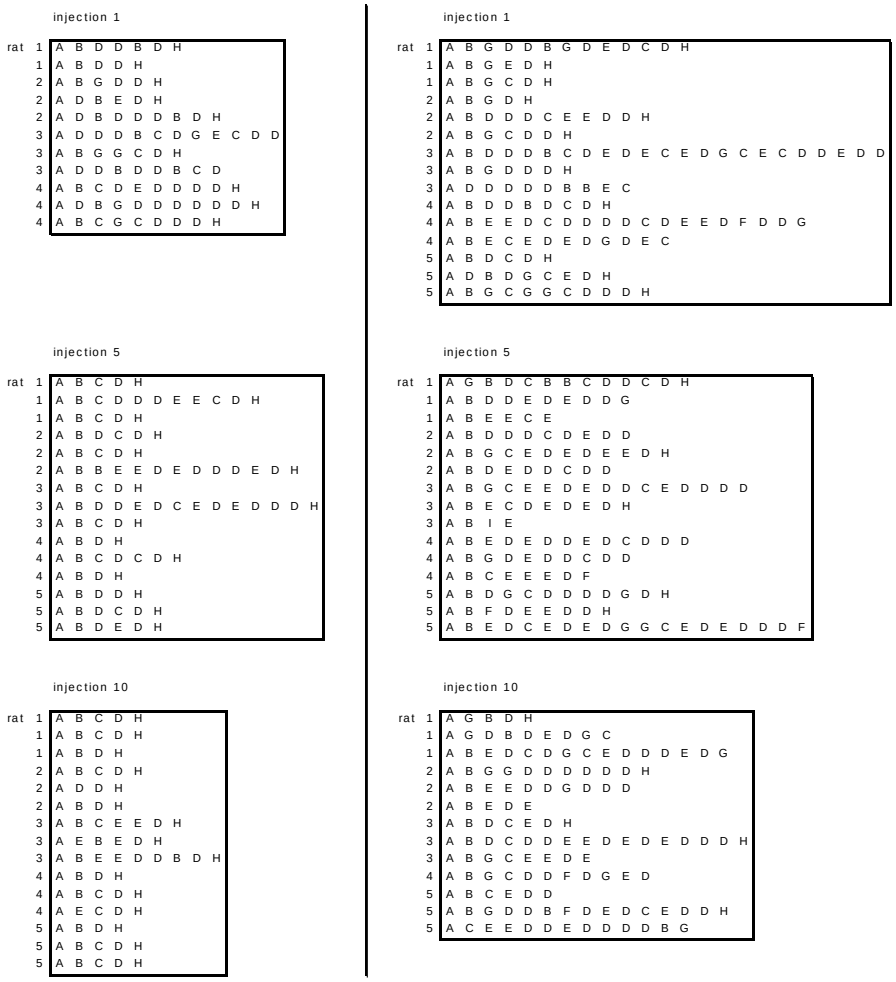
A: Saline-treated rat



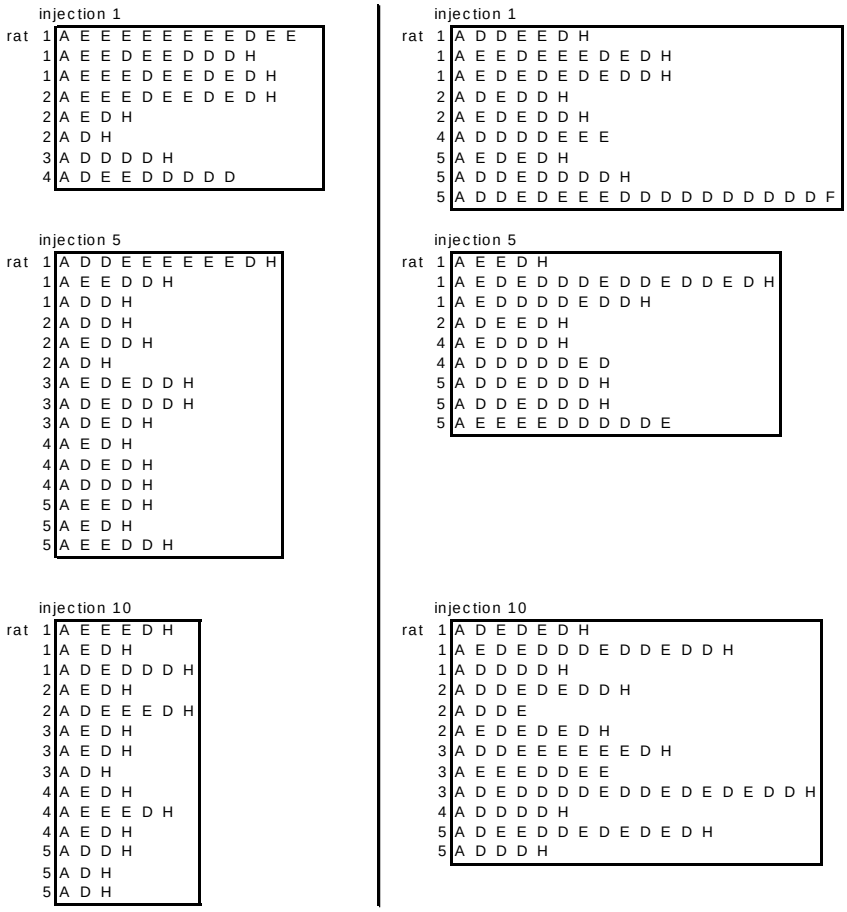
B: Quinpirole-treated rat



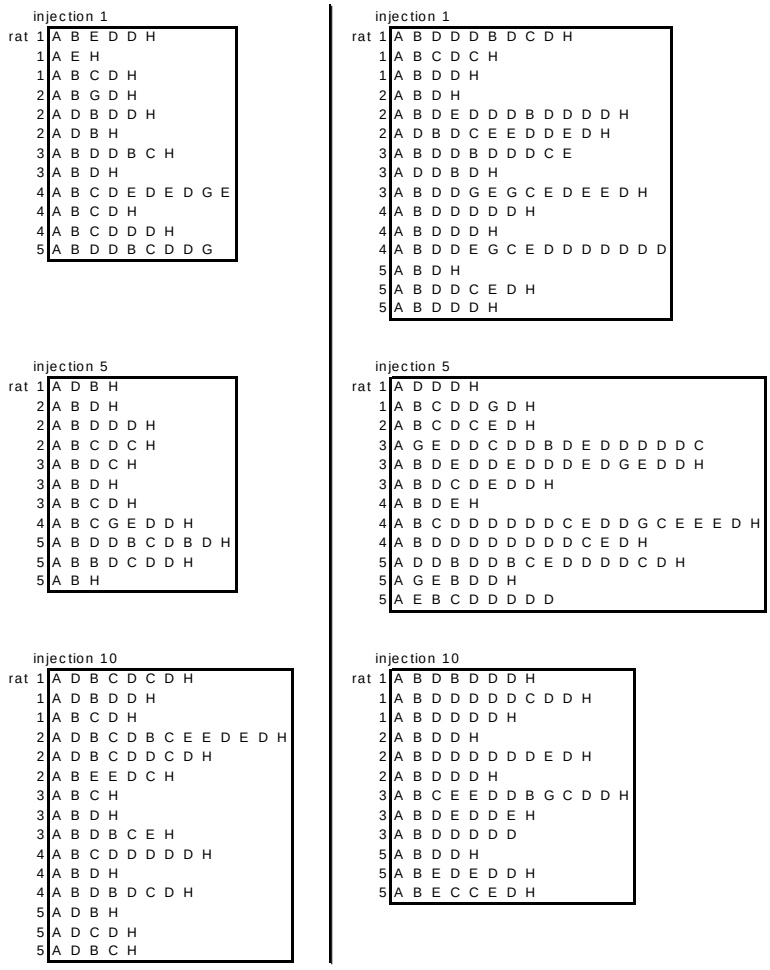
Eilam:Figure 4



Eilam: Figure 5



Eilam: Figure 6



Eilam: Figure 7