DIFFERENTIAL RECOVERY IN MEASURES OF EXPLORATION/LOCOMOTION AFTER A SINGLE DOSAGE OF RESERPINE IN THE RAT

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Abstract. Different aspects of exploration/locomotion were studied in rats injected with reserpine (4 mg/kg i.p.) once and in vehicle injected yoked controls. Fifteen different parameters were measured on 7 sessions — on the 1st, 3rd, 5th, 7th, 11th, 15th and 21st postinjection day. The "difference scores" (the value obtained from the control animal minus the corresponding value from the yoked reserpinized animal) were subjected to nonparametric analysis of variance across sessions. Of the 15 parameters only 6 revealed significant differences between sessions. Furthermore, for those six parameters which exhibited significant differences between sessions the "recovery patterns" revealed by differences between sessions were dissimilar. It is concluded that what is usually referred to as exploration/locomotion must be considered apparatus- and procedure-specific and that the measures obtained in different studies should only be compared when identical procedures have been employed. In addition to the measures of exploration/locomotion the rats were subjected to a parallel series of active avoidance sessions in a shuttle-box. Comparing the reserpinized rats' impaired active-avoidance acquisition to the recovery patterns in the exploration/locomotion tests, it can be tentatively concluded that the lack of recovery in the active-avoidance test is not solely due to impaired motoric abilities.

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INTRODUCTION

Many studies which investigate the behavioral consequences of pharmacological and/or surgical manipulations of the central nervous system include a measure of what is called exploration, locomotion, or "general activity". A number of techniques are used to measure this exploration/locomotion: open field, activity cage, running wheel, vertical hole-board etc. Usually, only a single technique is chosen for a given study and the result is reported to reflect either presence or absence of change in exploration/locomotion.

The use of the common term "exploration/locomotion" across the different testing methods may create the not necessarily correct impression, that a common neural substrate underlies the processes measured by the various techniques. Furthermore, valid comparisons between different studies, which have applied non-identical measuring techniques, would only by possible if, indeed, the various behaviors measured are mediated by a common neural substrate.

In the present study we have compared the ways in which different parameters of three tests of exploration/locomotion recover after a single i.p. injection of 4 mg/kg reserpine in the rat. Such a reserpine treatment is known to cause a relatively long-lasting monoamine-depletion (e.g. 2). The tests chosen were open field, activity cage and a newly developed vertical hole-board test (3-6). Additionally, the animals were subjected to an active shock-avoidance test.

If a common neural process was measured by all parameters of the exploration/locomotion tests, the behavioral recovery would appear at the same time and at the same rate in all variables. However, if measures of exploration/locomotion were mediated by at least not completely overlapping neural systems, the latter situation would indicate that exploration/locomotion should be seen as task-specific and only compared across studies if identical testing techniques were applied.

Although i.p. injection of reserpine was used to initiate the neural changes underlying the behavioral phenomena studied, a direct comparison with other studies using reserpine and measures of exploration/locomotion should be discouraged. The inclusion of the active avoidance test in the present study may have added to the neurochemical changes induced by reserpine (10). However, the exact nature of the induced neurochemical changes is not the main purpose of the present study — the topic presently investigated is whether or not various measures of exploration/locomotion are mediated by completely overlapping neural systems.
METHODS

Subjects. The subjects were 24 experimentally naive male Wistar-derived albino rats (from the rat colony at the Panum Institute), weighing approximately 300 g at the beginning of the experiment. They were housed in single cages with commercial rat chow and water always available. The animals' living quarters were maintained on a 12 h light/dark cycle (on 6.00 h; off 18.00 h). The rats were divided into 12 pairs each made of 2 animals of approximately equal initial weight. Within each pair the rats were randomly assigned to one of the two treatments groups: reserpine injection or vehicle injection control.

General procedure

On the day prior to injection all animals were weighed. On the injection day all reserpine animals received an i.p. injection of 4 mg/kg reserpine (200 mg reserpine was dissolved in 100 ml distilled water containing 250 mg citric acid monohydrate, 10 g polysorbate 80 and 100 mg parabenzoate) and all control animals received a vehicle injection. The animals were tested on the postinjection days 1, 3, 5, 7, 11, 15, and 21. The animals were injected at approximately 11.00 h and testing occurred between 8.00 and 13.00. On the test days, the animals were first subjected to a 15 min session of free exploration in the vertical hole-board apparatus. Immediately following this test the rat was transferred for 10 min into an open field. Following the open field session the rat was subjected to a 10 min activity cage session. Finally, the animal was given a 10 trial signaled two-way (step-through) shock-avoidance test.

Apparatus

Vertical hole-board. One semiopaque 8 mm thick wall in a 25.6 cm wide, 26.5 cm deep and 22.5 cm high opaque chamber had 54 1.7 cm diameter holes (arranged in 6 horizontal and 9 vertical lines); the 6 horizontal lines are referred to as “line A” to “F” — A being the top line, F the bottom line. The center to center distance between holes was horizontally and vertically 2.5 cm and diagonally 3.5 cm. The top of the box also served as door to the chamber. The floor of the chamber consisted of a wire grid. The wall containing the holes had a grid of 3 mm wide channels imbedded in it. Each channel had an infrared LED (light emitting diode) at one end and a photocell at the other end. The grid was arranged in such a way that each hole contained the crossing of one horizontal and one vertical line at its center. Nose-poking would break the infrared light beams of the two channels. The photocells were connected to an interface card through which the data were collected by
<table>
<thead>
<tr>
<th></th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Session 1</td>
</tr>
<tr>
<td>Hole board</td>
<td></td>
</tr>
<tr>
<td>No. of holes visited</td>
<td>16.5 (0-27)</td>
</tr>
<tr>
<td>Mean duration per visit</td>
<td>1.6 (1.0-2.0)</td>
</tr>
<tr>
<td>Total duration of visits to line:</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3.5 (0-21)</td>
</tr>
<tr>
<td>B</td>
<td>1.0 (0-7)</td>
</tr>
<tr>
<td>C</td>
<td>0.5 (0-10)</td>
</tr>
<tr>
<td>D</td>
<td>3.0 (0-6)</td>
</tr>
<tr>
<td>E</td>
<td>12.0 (0-22)</td>
</tr>
<tr>
<td>F</td>
<td>19.0 (0-33)</td>
</tr>
<tr>
<td>Open field</td>
<td></td>
</tr>
<tr>
<td>Latency to first movement</td>
<td>-600.0 (-600-600)</td>
</tr>
<tr>
<td>Total locomotion time</td>
<td>37.6 (8.1-159.4)</td>
</tr>
<tr>
<td>No. of locomotion episodes</td>
<td>19.0 (5-75)</td>
</tr>
</tbody>
</table>
### Table II

Measures of active avoidance performance as difference scores (scores of vehicle rat minus score of yoked reserpine rat)

<table>
<thead>
<tr>
<th></th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
<th>Session 4</th>
<th>Session 5</th>
<th>Session 6</th>
<th>Session 7</th>
<th>Anova</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active avoidance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of avoidances</td>
<td>0.0</td>
<td>0.0</td>
<td>1.5</td>
<td>2.0</td>
<td>5.5</td>
<td>6.0*+x</td>
<td>7.0+++</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(0-3)</td>
<td>(0-3)</td>
<td>(0-7)</td>
<td>(0-7)</td>
<td>(0-7)</td>
<td>(3-9)</td>
<td>(1-10)</td>
<td>(0-10)</td>
<td></td>
</tr>
<tr>
<td>No. of escapes</td>
<td>4.5</td>
<td>7.0</td>
<td>7.0</td>
<td>4.0</td>
<td>2.5</td>
<td>0.0x</td>
<td>0.5+++</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>(1-8)</td>
<td>(1-9)</td>
<td>(0-10)</td>
<td>(1-10)</td>
<td>(1-10)</td>
<td>(1-7)</td>
<td>(5-6)</td>
<td>(3-5)</td>
<td></td>
</tr>
</tbody>
</table>

* Significantly different from session 1 (P < 0.01); + Significantly different from session 2 (P < 0.01); * Significantly different from session 3 (P < 0.01)
RESULTS

The results are shown in Tables I and II in the form of median values (with range) for the difference scores.

The nonparametric analysis of variance demonstrated that for all three measures of exploration/locomotion, as well as for the active avoidance test, at least one parameter showed a recovery within the seven postinjection sessions. For the vertical hole-board the mean duration per visit and the total duration of visits in the two bottom “lines” (lines E and F) all showed significant recovery ($P < 0.01$), whereas no such effect was observed for the number of holes visited and the total duration of visits to the “lines” A-D. In the open field, the latency to first movement and the mean duration of locomotion episodes both demon-

![Graphs showing results](image_url)

Fig. 1. Performance of the two experimental groups on the aspects of exploration/locomotion which in the analysis of variance had been shown to contain significant session differences.
strated significant recovery \((P < 0.01)\), while such a recovery could not be demonstrated in the measures of total locomotion time, number of locomotion episodes, number of line-crossings and number of rearings. In the activity cage the only measure taken, "counts", demonstrated a significant \((P < 0.01)\) recovery.

Both measures obtained from the active avoidance test, the number of avoidances and escapes revealed significant session effects \((P < 0.01)\). However, while difference scores decreased in all measures of exploration/locomotion showing recovery, the trend of avoidance difference scores was just the opposite. In consequence, the significant session ef-

![Graph showing active avoidance performance](image)

**Fig. 2.** Active avoidance performance of the two experimental groups.

fect shown in the active avoidance test do not indicate "recovery", but rather an increasing group difference, namely, a gradual acquisition of the avoidance response by the vehicle treated animals and an impairment of such acquisition in the reserpine animals.

Tables I and II give additional details on the recovery profiles by
indicating significant between-session differences for those measures which showed significance in the nonparametric analysis of variance.

From the inspection of Table I it is obvious that the recovery profiles of the six parameters which did show a significant recovery within the seven sessions are dissimilar: while a significant difference \((P < 0.01)\) from session 1 could be seen already on the second session of the “counts” measure from activity cage (a parameter in which sessions 4-7 were also found to differ significantly \((P < 0.01)\) from session 1), the parameter total duration of visits to “line” E, at the other extreme, was found to have only session 6 significantly \((P < 0.01)\) different from session 1.

The significant \((P < 0.01)\) session differences for the two measures of active avoidance performance are shown in Table II.

Figure 1 and 2 show the actual performance of the experimental groups on the 6 aspects of exploration/locomotion, which in the analysis of variance had been shown to contain significant session differences and the two measures of active avoidance performance.

**DISCUSSION**

Out of a total of 15 parameters which all can be said to reflect one or another aspect of exploration/locomotion, only six demonstrated a significant recovery across the 7 postinjection sessions investigated in the present study. Furthermore, the recovery “profile” of those parameters, which did demonstrate a behavioral recovery after reserpine treatment, varied between parameters. These findings seem to indicate that the neural processes which mediate the different response components are at least not completely overlapping. Therefore, exploration/locomotion must be considered task-specific and component-specific within a given task, to such an extent that it is not possible directly to compare measures obtained with different procedures and apparatuses.

The above conclusion is independent of the actual causes of the “profile” changes observed over time — although named “recovery”, the interpretation in terms of non-overlapping neural substrates would hold whether or not recovery is contributing significantly to the changes. However, as can be seen from Fig. 1, in all 6 parameters shown the reserpinized animals exhibited recovery-like changes of performance.

Although the present data allow us to conclude that the neural processes mediating the different aspects of exploration/locomotion are not completely overlapping, the nature of this difference between systems is still obscure. They may be situated in different structures as defined
by classical neuroanatomy, they may be represented by different transmitter systems within the same structure, or both "structural" and "neurochemical" differences may exist.

It could be speculated that the impaired acquisition of active avoidance seen in the reserpinized animals might be, at least partly, due to "learned helplessness" (10) or to some other type of proactive effect of the increased shock exposure resulting from escape failures in the initial sessions. The notion that a purely motoric disturbance is the sole reason for the learning impairment of the reserpine rats is not supported by our data, since the active avoidance behavior of the reserpine group hardly shows any improvement across sessions (Table II and Fig. 2) while several measures of locomotion (e.g. the counts in the activity cage) (Table I and Fig. 1) demonstrate considerable recovery across sessions.

The present findings, like those obtained in other studies comparing different versions of formally the same test (e.g. 1, 7 and 8), emphasize the necessity of stating the exact technique used to measure a certain behavioral phenomenon, and the possible pitfalls of generalizations made across studies, if differences between measuring techniques are not taken into account.

It should be emphasized that the observed differences between the reserpine treated animals and the vehicle injected yoked controls are not necessarily a reflection of the neurochemical changes induced by reserpine only: all the animals were subjected to the active avoidance test and the reserpinized animals received substantially more shocks than the controls. It must be considered likely, therefore, that the reserpine animals suffered greater shock-induced neurochemical changes (e.g. further depletion of central norepinephrine (10)) relative to the vehicle injected animals. These reservations, however, do not subtract from the basic finding of a differentiation between the employed measures of exploration/locomotion: whatever the cause of neuronal changes, all parameters were measured on the same days on the same animals and did nevertheless exhibit different changes over time.

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