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Effects of Scopolamine, d-Amphetamine, and Apomorphine on Alternation and Position Biases

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McFARLAND, D. J. *Effects of scopolamine, d-amphetamine, and apomorphine on alternation and position biases.* PHARMACOL BIOCHEM BEHAV **32**(3) 723–726, 1989.—The sequencing of arm entries in a symmetrical Y-maze was examined in mice treated with either scopolamine, d-amphetamine or apomorphine. These treatments could potentially alter both alternation tendencies and rotational tendencies. Therefore, a measure of spontaneous alternation was evaluated which was computed by taking an average of the right going percent alternation and the left going percent alternation. The number of arm entries was increased by amphetamine, unchanged following scopolamine and reduced by apomorphine. All three drugs reduced spontaneous alternation tendencies and increased the magnitude of a bias to turn consistently in the same direction (rotational tendency). All three drugs thus altered the sequencing of arm entries from patterns consistent with spatial alteration to patterns consistent with egocentrically defined responses. These results indicate that the measure of spontaneous alternation which was an average of the right going percent alternation and the left going percent alternation is a better index of alternation tendencies. Thus, when an animal is not able to navigate on the basis of an extrapersonal (allocentric) system as a result of drug treatment, it will revert to an egocentric system.

Spontaneous alternation Amphetamine Scopolamine Apomorphine

SPONTANEOUS alternation refers to the tendency of normal animals to visit the least recently entered or experienced arm of a maze. The phenomenon of spontaneous alternation was described by Tolman (21) as an example of hypothesis behavior in rats. This was considered to be the lose-shift component of a win-stay, lose-shift hypothesis. Alternative explanations of this phenomena have included inhibitory tendencies, stimulus seeking (5) and memory (11). While the original example was based on observations from a T-maze discrete trial paradigm, subsequent studies demonstrated spontaneous alternation in a variety of situations (6). Spontaneous alternation has been used for the behavioral assessment of a variety of drugs including catecholamine stimulants and anticholinergic agents (20).

Two procedures are commonly used in the study of spontaneous alternation. The first involves the use of a discrete trial procedure in a T-maze (10) and the second involves the use of a continuous Y-maze procedure (9). Given that an animal makes a left turn on trial N-1, a right turn on trial N would represent an alternation response in the discrete trial situation. The fact that a bias towards turning in one direction will compete with alternation tendencies has been recognized for some time (16) and methods for correction of this bias have been proposed (7).

Alternation behavior in the discrete trial paradigm could represent either an alternation of responses with respect to an egocentric orientation (i.e., left vs. right) or an alternation of places in extrapersonal space. It has been suggested that the results obtained with the continuous Y-maze procedure support the concept that normal animals tend to alternate places rather than egocentrically defined responses (9). With this procedure an animal is allowed to freely explore a symmetrical Y-maze. If the animal turns right on both trial N-1 and trial N, in this paradigm, the trial N response is considered to be an alternation and represents a visit to the least recently entered arm of the maze.

In the continuous Y-maze paradigm an alternation response as generally scored also represents two consecutive turns in the same direction. If right and left turns are represented by R and L respectively, and the allocentric position of the arms are numbered clockwise, then the sequences 1 2 3 1 and L L L are identical in the continuous Y-maze. These represent perfect alternation and a perfect left going position bias (note that one additional symbol is needed for the allocentric notation system to denote the arm from which the animal starts). The identity of these sequences illustrates that alternation and position bias are related at a formal (mathematical) level. Likewise, the sequences L R L R and 1 2 1 2 are identical in the continuous Y-maze and represent both avoidance of arm 3 and egocentric response alternation. Fortunately, animals seldom exhibit perfect alternation or bias tendencies so that it is possible to compute allocentric alternation scores which are the average of left going and right going alternations.

Since alternations in the continuous Y-maze paradigm are also consecutive turns in the same direction, an animal with no true alternation tendency and a bias to turn in a given direction 80% of the time would be expected to show "alternation" on 68% of the trials since the probability of two consecutive responses in the same direction would be $(0.8 \times 0.8) + (0.2 \times 0.2)$. There are thus two problems in determining how best to characterize the animal's

behavior. The first is whether the animal's behavior is best characterized (or consistent with) alternation as opposed to perseveration or preferences. The second is whether the patterning of behavior is more consistent with an orientation based on the animal's own body-axis (egocentric) or on external landmarks (allocentric). These two types of tendencies need to be considered simultaneously since in a situation such as the continuous Y-maze, allocentric alternation appears superficially to be the same as egocentrically defined perseveration.

Certain drugs such as d-amphetamine are known to cause rotation in normal animals (12). Kokkinidis and Anisman (14) compared the effects of d-amphetamine injected intraperitoneally and intraventricularly on performance in a continuous Y-maze and in a circular alley. Both routes of administration produced circling behavior in the circular alley. Since intraperitoneal administration reduced alternation it was concluded that systemic d-amphetamine normally produces perseveration in a Y-maze. The possibility that both perseveration and rotation effects could occur simultaneously was not considered.

The present study was concerned with simultaneously examining position bias and alternation responses in animals given drugs which affect both of those tendencies. This was done by recording whether or not an alternation or nonalternation response was also a left or right going response. This allowed for the computation of percent alternation separately for left and right going responses. The average of these two produces a measure of alternation which is not inherently correlated with position biases at a formal level.

METHOD

All animals were housed in plastic suspended cages in groups of three with food and water available ad lib. The room was maintained on a 12-hr on, 12-hr off lighting schedule with all testing conducted during the light phase. In both experiments the subjects were 8–9-week-old female Nya:NYLAR mice obtained from the New York State Health Department breeding facilities at 3 weeks of age. Each drug treatment group included 12 mice not previously employed in any other experiment. The apparatus was a $27.5 \times 7.5 \times 10$ cm symmetrical Plexiglas Y-maze painted flat black. Photocells, illuminated by infrared LEDs, were located 10 cm within each arm and 2.5 cm from the floor. The mouse was placed at the far end of one arm and the maze was covered. Data was recorded during a 10-min session.

The sequence of arm entries was recorded with the aid of an ACT/Interact laboratory computer system. The system was programmed not to count two or more successive breakings of the same photocell. The frequency of alternations (least-recently entered arm) and nonalternations on a given trial were recorded in separate categories depending upon whether the response represented a left turn or right turn. Alternation for each separate category (right vs. left turns) was computed as the frequency of alternations divided by the total frequency of responses for that category. This proportion was multiplied by 100 to produce percentage scores. This classification of responses can apply only on those arm entries preceded by at least two previous arm entries. The corrected alternation score consisted of the average of right going alternations divided by the total number of right turns and left going alternations divided by left turns. This gave an average alternation score which was not biased by a tendency to turn more often than not to a given direction. A measure of position bias was computed as the absolute value of the difference between right turns and one-half of the total turns. The resulting value was divided by the total number of arms entered multiplied by 100 and then added to 50 so as to give the percentage of responses in the preferred direction. All measures were computed individually for each animal.

TABLE 1

EFFECTS OF d-AMPHETAMINE AND SCOPOLAMINE ON TOTAL ALTERNATION, CORRECTED ALTERNATION (AVERAGE OF LEFT AND RIGHT GOING PERCENT ALTERNATIONS) AND POSITION BIAS (PERCENT SELECTION OF FAVORED DIRECTION)

	Means				Correlations With Total Alternation	
Dose of Drug	Arms Entered	Total Alternation	Corrected Alternation	Position Bias	Corrected Alternation	Position Bias
Saline	57.8	70.3	69.3	57.2	+.99†	−.05
Amphetamine (3 mg/kg)	125.1†	66.3	63.9	61.2	+.96†	+.51
Amphetamine (6 mg/kg)	106.5†	61.2	53.3†	72.2†	+.91†	+.48
Scopolamine (3 mg/kg)	70.3	57.2	52.2†	64.2	+.65*	+.76†
Scopolamine (6 mg/kg)	66.1	61.9	52.4‡	68.2†	−.08	+.62*

*$p<0.05$: for means, the difference between saline and drug treatment; for correlations the null hypothesis of no relationship.
†$p<0.01$: as above.

The effects of d-amphetamine and scopolamine were examined in Experiment 1. These drugs were dissolved in sterile saline at a concentration such that each animal received a volume of 4 ml/kg. All drug solutions were prepared fresh daily. Separate groups of animals received either 3 mg/kg or 6 mg/kg of d-amphetamine sulfate (Smith, Kline and French), 3 mg/kg or 6 mg/kg scopolamine hydrobromide (Aldrich Chemical Co.) or saline. All injections were given IP 10 minutes prior to the start of testing.

The effects of apomorphine hydrochloride (Merck, Sharp and Dohme) were examined in a second study. This drug was dissolved in sterile saline at a concentration such that each animal received a volume of 4 ml/kg. All solutions were prepared fresh daily. Separate groups of animals received either saline, 0.75 mg/kg of 1.50 mg/kg IP 10 minutes prior to the start of testing.

RESULTS

Table 1 contains a summary of total alternation scores, corrected alternation scores (average of left and right going percent alternation) and position bias scores (percent selection of the preferred side) for both amphetamine and scopolamine treatments.

Analysis of variance indicated that the effect of drug treatment on the total alternation score was not significant. An analysis of variance indicated that the corrected alternation score was significant, $F(4,55) = 5.88$, $p<0.001$, with the 6 mg/kg dose of amphetamine and both scopolamine doses differing from controls ($p<0.01$ in all cases). The effect of drug treatment on the number of arms entered was significant, $F(4,55) = 12.71$, $p<0.001$, with animals given both amphetamine doses entering significantly more arms ($p<0.01$ in both cases). Finally, the effect of drug treatment on the measure of position bias was significant, $F(4,55) = 3.46$, $p<0.05$, with both the 6 mg/kg dose of amphetamine and the 6 mg/kg dose of scopolamine showing an increased bias towards one side ($p<0.01$ in both cases). Thus, while amphetamine and scopolamine had different effects on activity, both reduced the level of spontaneous alternation as measured by the corrected score and increased the position bias of the animals.

Also included in Table 1 are correlations between the total alternation score, the corrected alternation score and the measure

TABLE 2

EFFECTS OF APOMORPHINE ON TOTAL ALTERNATION, CORRECTED ALTERNATION (AVERAGE OF LEFT AND RIGHT GOING PERCENT ALTERNATION) AND POSITION BIAS (PERCENT SELECTION OF FAVORED DIRECTION)

		Means			Correlations With Total Alternation	
Dose of Drug	Arms Entered	Total Alternation	Corrected Alternation	Position Bias	Corrected Alternation	Position Bias
Saline	47.7	73.0	69.8	61.8	+.85†	−.32
Apomorphine (0.75 mg/kg)	17.2†	65.6	56.8*	70.1	+.79†	+.31
Apomorphine (1.50 mg/kg)	15.9†	57.0†	44.7†	75.7†	+.77†	+.79†

*$p<0.05$: for means, the difference between saline and drug treatment; for correlations the null hypothesis of no relationship.

†$p<0.01$: as above.

of position bias. Of interest here is the fact that with saline and both amphetamine doses the total alternation score was related mainly to the factor measured by the corrected alternation score. Given scopolamine however, the relative contribution of the factor measured by the corrected alternation score decreased and the correlation between total alternation and position bias increased.

Table 2 contains a summary of total alternation scores, corrected alternation scores (average of right and left going alternation tendencies) and response bias scores. Analyses of variance indicated that the effects of apomorphine on alternation, $F(2,33)=3.78$, $p<0.05$, the corrected alternation score, $F(2,33)=11.2$, $p<0.001$, and the number of arms entered, $F(2,33)=62.5$, $p<0.001$, were all significant. The total alternation score was depressed by the high dose of apomorphine ($p<0.01$). The corrected alternation score was depressed by both doses of apomorphine ($p<0.05$ in both cases). Only the 1.5 mg/kg dose of apomorphine resulted in a significant increase in the measure of position bias ($p<0.01$). Total alternation scores were significantly correlated with corrected alternation scores under all conditions while the position bias score and total alternation score were significantly correlated only in the 1.5 mg/kg apomorphine group. The number of arms entered was significantly depressed following both doses of apomorphine. Thus, apomorphine, like both amphetamine and scopolamine, reduced alternation levels while simultaneously increasing the magnitude of the position bias.

DISCUSSION

The results of both studies indicate that the role of position biases should be examined when the action of psychotropic agents and other treatments are evaluated with the continuous Y-maze alternation paradigm. This procedure can provide simultaneous measurement of three independent behavioral tendencies: alternation, position biases and the number of arms entered. That these measures are independent is indicated by the fact that d-amphetamine increased activity levels, scopolamine had no significant effect on activity and apomorphine depressed activity levels. All of these agents reduced alternation levels and increased the magnitude of the position bias. Thus, while the corrected alternation score proposed here is independent of the position bias measure at a formal level, at least in the cases observed here, it is negatively correlated empirically (biologically). This indicates that when these drug treatments reduce allocentric alternation tendencies, egocentric position biases emerge. This explanation of the action of these drugs is consistent with the interpretation of the effects of cholinergic receptor blockade (23) where it is suggested that locale systems (allocentric) and not taxon systems (egocentric) are affected by atropine.

The fact that alternation and position biases covary in opposite directions in response to these drugs suggests one of two possible effects. The tendency towards an egocentric position bias may be increased and compete with alternation tendencies. Alternatively, the alternation tendency may be reduced and the use of the position bias as the predominant response tendency would then emerge. It is also possible that these two response tendencies could be differentially affected by different drugs. Since what is measured is in effect only the extent to which behavior is consistent with a given response tendency, these two alternatives cannot be discriminated between in this situation.

It has been suggested that the effects of amphetamine on spontaneous alternation are qualitatively different from those of scopolamine (2,13). This is based in part upon the observations that scopolamine reduces alternation to chance levels while amphetamine produces perseverative (below chance) behavior. This difference is interpreted as indicating that anticholinergics reduce habituation while amphetamine increases responding to selective stimuli. Alternation is then thought of as resulting from selective inhibition (habituation) of responses to nonreinforced stimuli (3). One problem with this formulation is that alternation levels significantly below chance have been observed following high doses of scopolamine (10). In the present study the effects of 6 mg/kg of amphetamine and 6 mg/kg of scopolamine are very similar with respect to the total alternation score, the corrected alternation score and the measure of position bias. However, it should be noted that factors such as exposure to the maze (13) or the time since drug administration may influence the effects observed.

Amphetamine, apomorphine and scopolamine changed the animal's behavior from a pattern consistent with allocentric spatial alternation to one consistent with egocentric position bias. This observation is consistent with an increase in responding to selective stimuli to the extent that such stimuli are those that guide egocentric orientation. Alternative interpretations are possible. For example, several investigations have used spontaneous alternation behavior as an index of memory functioning (19,24). The present results suggest that if memory were disrupted by amphetamine and scopolamine then it would be allocentric spatial memory which is affected. The present results do not allow for a discrimination between these alternative hypotheses but merely suggest that drug treatment causes a shift from behavior consistent with allocentric alternation to behavior consistent with an egocentric bias.

Current research suggests that the hippocampus is an important component of the neural circuitry which deals with allocentric spatial information (17). Likewise, the caudate nucleus has been implicated as a component of the neural circuitry which processes egocentric spatial information (1,4). Although cholinergic afferents from the magnocellular basal nucleus project diffusely to telencephalic structures (18) the net effect of drugs which block muscarinic cholinergic receptors often resembles that of lesions of the hippocampus (8). In particular, scopolamine disrupts performance on a radial maze task in a manner similar to that of hippocampal lesions (22). The net effect of dopaminergic agonists on postural asymmetry has been related to nigro-striatal function (12). It thus seems likely that ascending dopaminergic and cholinergic systems modulate telencephalic structures which process egocentric and allocentric information.

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