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Aggression Increase and Water Competition Decrease in Squirrel Monkeys Given Physostigmine Injections[1]

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THOMAS, G. S., D. J. CACCAMISE AND D. L. CLARK. *Aggression increase and water competition decrease in squirrel monkeys given physostigmine injections.* PHARMAC. BIOCHEM. BEHAV. 8(6) 633–639, 1978. – Six adult squirrel monkeys *(Samiri sciureus)* were tested in pairs for social dominance in a water competition task. Dominance was defined by two methods: (1) number and direction of aggressive responses, and (2) successful water competition as assessed by latency to the water bottle, latency to accumulate 15 seconds of drinking, and total drinking duration. Monkeys were assigned to pairs on a "round robin" basis so that each monkey was paired with all other monkeys under control, saline, and three levels of physostigmine sulfate (6.25, 12.5, and 25.0 μg./kg.). Under drug conditions only one member of each pair was drugged. The 12.5 μg./kg. dose of physostigmine resulted in a significant increase in aggressive responses. Both drinking duration and general motor activity decreased with increasing dose level of drug. It was also noted that the non-drugged partners of drugged monkeys accumulated 15 seconds of drinking faster and drank more at the 25 and 12.5 μg./kg. dose levels than under control and saline conditions. Physostigmine resulted in an increase in one measure of social dominance and a decrease in the other.

Aggression Drug-induced aggression Physostigmine Social dominance Squirrel monkey Water competition

THE CENTRAL cholinergic system has been implicated in the mediation of a variety of behaviors in infrahumans. Cholinergic and anti-cholinergic drugs have been shown to affect general motor activity, food and water intake, discrimination and avoidance learning, memory, and social-emotional and aggressive behaviors.

The effects of cholinergic and anti-cholinergic drugs on aggression have been investigated using shock-induced aggression and muricide paradigms. Anti-cholinergic drugs such as scopolamine and atropine inhibit shock-induced aggression [13,20], whereas, a facilitation in shock-induced aggression has been demonstrated with cholinergic stimulants [21]. Cholinergic and anti-cholinergic drugs also differentially affect muricide. Anti-cholinergic drugs inhibit [12], whereas, cholinergic drugs increase the occurrence of muricide [2, 7, 9, 15, 24, 25].

The above mentioned studies typically used rats as subjects; however, spontaneous aggression in squirrel monkeys was investigated in competitive social situations using an anti-chlinergic drug. The drug, scopolamine, was found to generally depress aggressive responses and food getting behaviors of the monkeys [19].

Direct central cholinergic stimulation has been found to increase drinking in both sated and water-deprived animals. In a recent review of the literature [8], cholinergic neurons in a variety of brain structures were implicated in the mediation of thirst through the use of intracranial injections of cholinergic drugs. Conversely, it has also been reported that peripheral injections of cholinergic stimulants, such as physostigmine and DFP, resulted in decreased drinking in both water-deprived and non-deprived rats [1, 10, 11].

The present study was conducted to investigate the effects of the cholinergic stimulant physostigmine on aggressive behavior and three competitive behavioral measures of social dominance.

Stable linear dominance hierarchies in squirrel monkeys have been defined in terms of the frequency and direction of aggressive responses in competitive social situations [18]. Monkeys, which emitted more aggressive responses than they received, were defined as dominant. Stable hierarchies in squirrel monkeys were also observed with control of access to food and water incentives as criteria for dominance [6,16]. In addition, high correlations have been reported between measures of dominance based upon control of access to water and measures of dominance based on aggressive responses in squirrel monkeys and pigtail macaques [4,5]. For example, Clark and Dillon [5] identified two measures which correlated highly with measures of aggressive social dominance: Total drinking duration and latency to accumulate 15 sec of drinking. The current study employed an apparatus and procedure similar to Clark and Dillon [5] to assess the effects of physostigmine on two commonly used and highly correlated

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measures of social dominance, water competition and aggressive behaviors.

METHOD

Animals

Six feral reared adult male squirrel monkeys *(Saimiri sciureus)*, weighing from 650–980 g, were maintained on ad lib feeding and housed individually between experimental sessions. Animals were water deprived for 12 hr prior to experimental sessions.

Apparatus

Animals were observed in pairs in a chamber measuring 75 x 40 x 70 cm. The side facing the observation station was plate glass, and two adjacent entry hatches allowed simultaneous entry of both animals into the chamber. A single retractable drinking spout protruded into the chamber and was attached to a reservoir containing 100 ml of Hawaiian punch, with a concentration of one part punch to eight parts water.

Procedure

During the study, three pairs of monkeys were tested once every other day, three test days per week. Pairings were assigned according to a "round robin" schedule so that each monkey was paired with all other monkeys. Experimental conditions were assigned to test days in the sequence: saline, drug, control, drug. This sequence was repeated throughout the study. On saline days both members of each pair were chaired and injected with saline 45 min prior to testing. Control days excluded all injection procedures prior to testing. On drug days, one member of each pair received a drug injection and the other received a saline injection 45 min prior to testing. All pair combinations were repeated until each animal had been injected with each of the three drug doses when paired with all other monkeys. Drug dose level was randomly assigned to monkeys with the constraints that only one monkey of any given pair was drugged, and that no monkey received drugs more than once in 96 hr or twice in any given week.

The test procedure consisted of the following: at the end of five sec buzzer signaling the onset of the trial, both animals were simultaneously admitted to the test chamber where the drinking spout was readily available. Five one-min periods of drinking were alternated with five one-min periods of no access to the water bottle for a total trial time of 10 min. The onset of the drinking availability periods and the nonavailability periods was signaled by a five sec buzzer and a five sec high pitched tone, respectively.

Measures

Cumulative time measures included: *latency to the water bottle:* the time it took for a monkey to take its first drink; *15 sec latency:* the time it took a monkey to accumulate 15 sec of drinking; *drinking duration:* the total amount of time a monkey spent drinking; and *general motor activity:* defined as simultaneous movement of two limbs resulting in a 2 cm movement of the monkey's torso. The drinking measures were taken from Clark and Dillon [5].

Social aggressive measures, taken from Plotnik, King, and Roberts [18], included: grabs, pushes, bites, chases, displacements, and genital displays. In addition, avoidance responses, defined as the movement of one body length away from an approaching monkey, were also recorded.

Response measures were recorded by two observers, each with a set of cumulative frequency counters, and each responsible for recording behaviors of one of the animal pairs. Cumulative time measures (e.g., the water competition measures) for pre-experimental individual testing had inter-rater reliabilities of from .999 to .987. These reliabilites were calculated by correlating the drinking data obtained on the monkeys by the two observers. Social interactions (e.g., aggression and avoidance responses) between monkeys in paired testing were few enough that both observers could agree as to the appropriate response classification before the data was recorded.

Observers remained blind as to drug and saline conditions throughout the study. Drug and saline injections were administered to the monkeys by the senior author before the animals were delivered to the observers. The drug injection schedule was not revealed to the observers until after the entire experiment was completed.

Pre-Experimental Training

The monkeys were trained to drink from the water spout, trained to compete for water in pairs, and adapted to injection procedures in three phases: (1) The monkeys were placed individually into the test chamber daily until each accumulated, on three consecutive days, 15 sec of drinking within the first min of drinking access and 240 sec of drinking in five min. (2) Monkeys competed in pairs for water on a "round robin" basis until each monkey had been paired three times with all other monkeys. Each animal was observed once a day until all pairings were complete. (3) The last stage of training included all aspects of the testing procedure with the exclusion of drug injections. Prior to social testing, each monkey was chaired and injected with saline. They were then placed in the test situation in pairs until all possible pairings were complete. Animals were tested only once every other day. Previous research, Thomas (unpublished Master's Thesis, 1975), indicated that handling and restraint necessary for injections did not disrupt social interactions.

Hierarchy Assessment

The dominance hierarchy was determined during the final stages of training before the introduction of drugs to the study. The hierarchy was based on aggressive responses and competitive drinking behaviors recorded during the final two "round robin" pairings of pre-experimental training. Based on aggressive responses, a monkey's position in the dominance order was determined by the ratio of aggressive responses emitted to those received from each other monkey. The greater the ratio, the higher was the monkey's position in the dominance hierarchy. An identical dominance order was observed based upon the 15 sec latency and drinking duration measures. Monkeys which accumulated 15 sec drinking faster and drank longest were considered the dominant members of the pairs.

The above procedures allowed a rank ordering of the monkeys based on the social dominance measures. For purposes of subsequent data analyses the three highest ranking monkeys were designated as dominant and the three lowest ranking monkeys were designated as submissive.

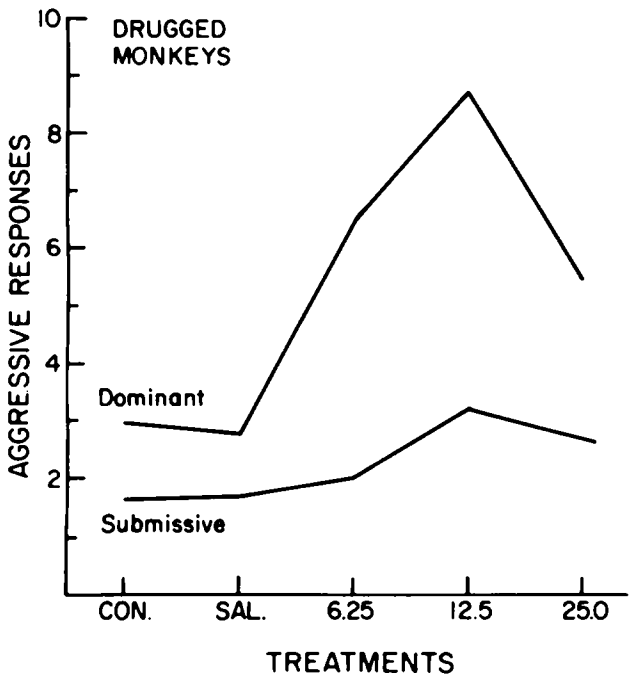

FIG. 1a. Mean number of aggressive responses per test session of drugged, dominant and submissive monkeys under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

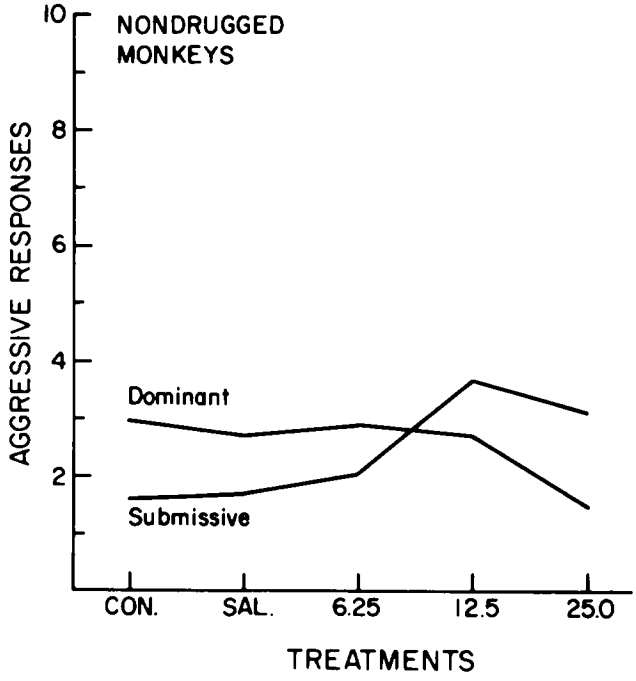

FIG. 1b. Mean number of aggressive responses per test session of dominant and submissive, non-drugged partners of drugged monkeys which were under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

Drug

Physostigmine sulfate was dissolved in distilled water and administered in doses of 6.25, 12.5, or 25 μg/kg. All injections were administered SC in a volume of 1 ml/kg. Drug and saline injections were timed to occur 45 min prior to testing to obtain maximum drug effects. Pilot testing of the drug indicated that the maximum decrease in heart rate, to a rate of 200 beats per min, occurred at 45 min after injection of 12.5 μg/kg physostigmine.

RESULTS

Although the dominance hierarchy remained stable throughout the study, the number of aggressive responses initiated by each monkey varied as a function of physostigmine injections. As indicated in Fig. 1a, the number of aggressive responses of drugged monkeys increased with respect to their saline and control levels of behavior.

For statistical comparison, each monkey's number of aggressive responses were averaged across encounters with each other monkey for each of the five experimental conditions: saline, control, and the three levels of drug. These data were cast into a 2 × 5 repeated measures ANOVA where the three highest ranked monkeys were considered dominant and the three lowest ranked considered submissive. (The same procedure was used in all subsequent analyses.) The current analysis and post-hoc Tukey's HSD Test indicated that the 12.5 μg/kg dose of physostigmine produced a significant increase in aggressive responses as compared to saline and control conditions, $F(4,16) = 3.97$, $p < 0.025$; 12.5 μg/kg>control and saline, $p < 0.01$.

Although physostigmine appeared to have a greater affect on dominant monkeys than on submissive monkeys, this was not supported by statistical analyses. Dominant monkeys were not significantly more aggressive than submissive monkeys, $F(1,4) = 3.69$, and drug injections did not significantly interact with dominance order, $F(4,16) = 1.61$.

As presented in Fig. 1b, the number of aggressive responses initiated by non-drugged monkeys toward their drugged partners varied little across experimental conditions. A 2 × 5 ANOVA indicated that the number of aggressive responses by non-drugged monkeys did not differ across experimental conditions, $F(4,16) < 1.0$. In addition, non-drugged dominant monkeys did not differ in number of aggressive responses from non-drugged submissive monkeys, $F(1,4) < 1.0$, and the interaction of experimental condition-by-dominance was not significant, $F(4,16) = 2.68$.

Baseline number of avoidance responses were low and did not change as a result of drug injections. An ANOVA comparing the number of avoidance responses of non-drugged monkeys in reaction to their drugged partners did not differ significantly across experimental conditions, $F(4,16) < 1.0$.

Although drugged monkeys tended to be more aggressive, their general motor activity tended to decline with physostigmine injections. As presented in Fig. 2a, general activity decreased linearly as dose level increased. An ANOVA and Tukey's HSD test indicated that the 25 μg/kg dose level resulted in less general motor activity than activity observed under saline, control, or 6.25 μg/kg of drug conditions, $F(4,16) = 4.69$, $p < 0.025$; 25 μg/kg>saline, control, and 6.25 μg/kg drug, $p < 0.05$. As indicated in Fig. 2b the motor activity of the non-drugged partners of drugged monkeys increased only when drugged monkeys

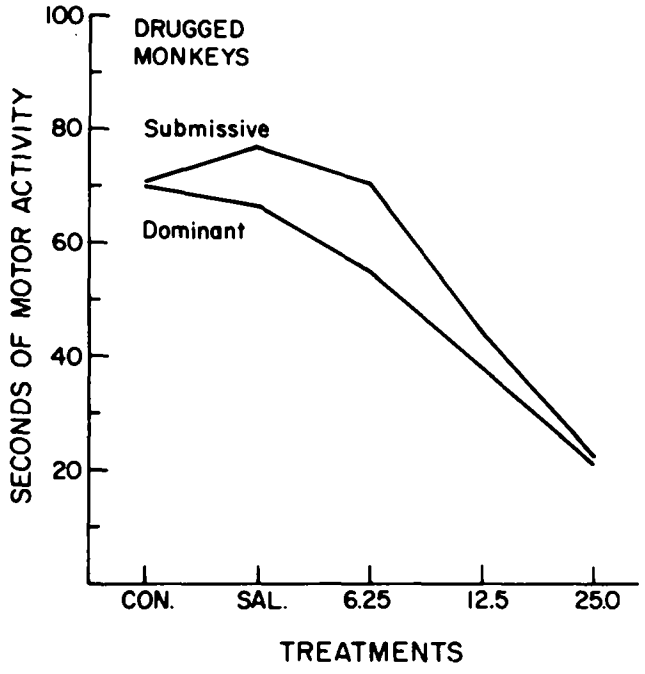

FIG. 2a. Mean number of sec of general motor activity per test session of drugged, dominant and submissive monkeys under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

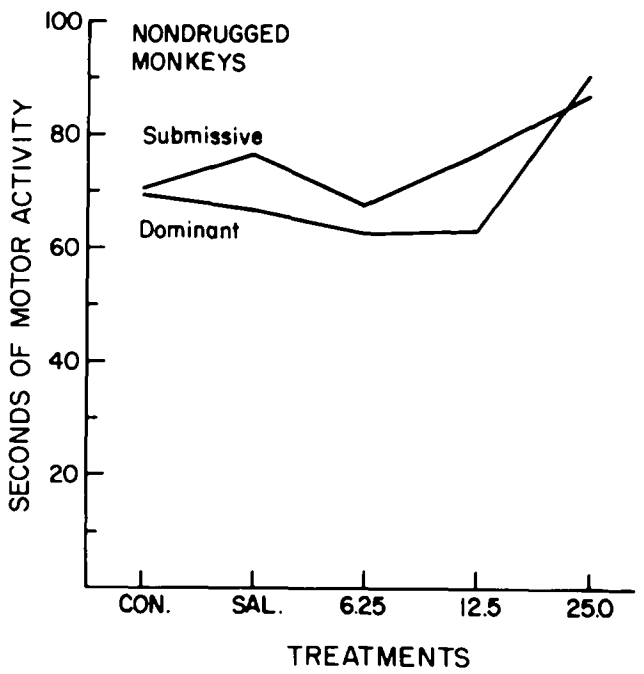

FIG. 2b. Mean number of sec of general motor activity per test session for dominant and submissive, non-drugged partners of drugged monkeys which were under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

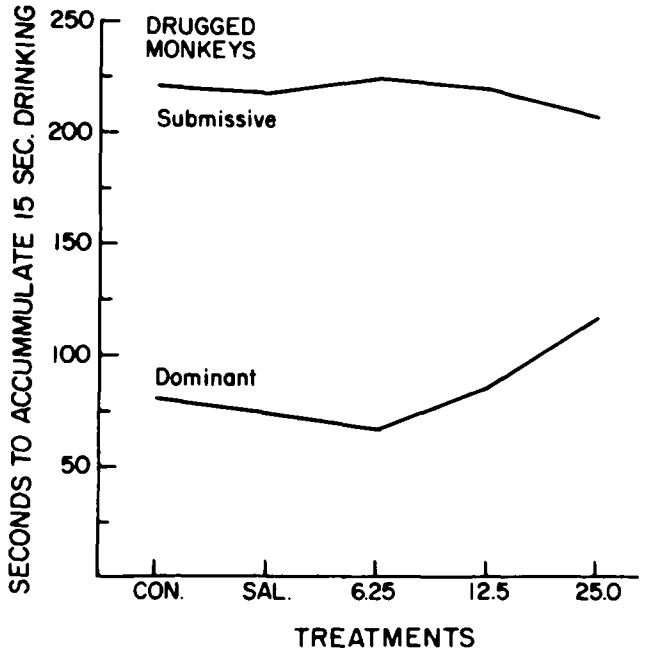

FIG. 3a. Mean number of sec latency to accumulate 15 sec drinking per test session for drugged, dominant and submissive monkeys under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

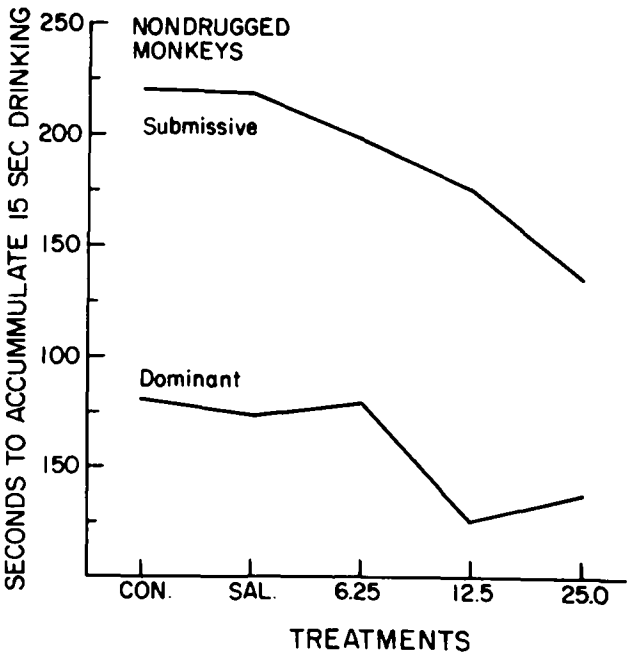

FIG. 3b. Mean number of seconds latency to accumulate 15 sec drinking per test session for dominant and submissive, non-drugged partners of drugged monkeys which were under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

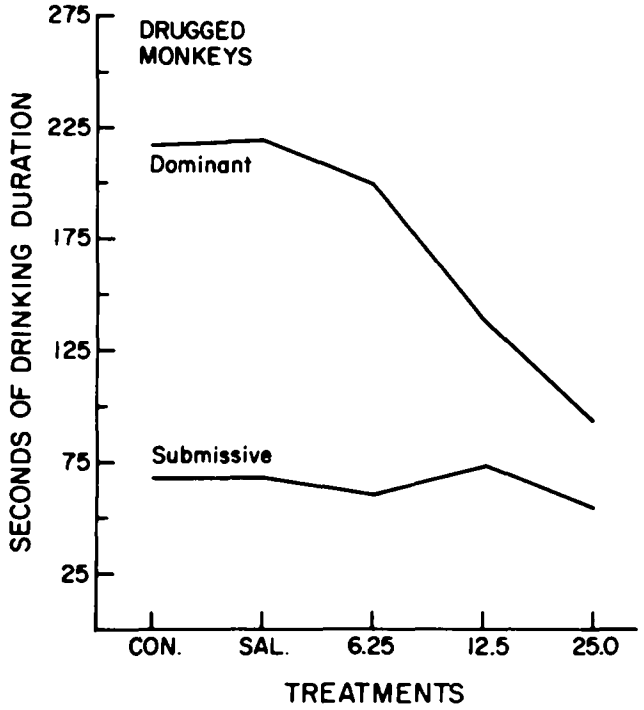

FIG. 4a. Mean number of seconds drinking duration per test session of drugged, dominant and submissive monkeys under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

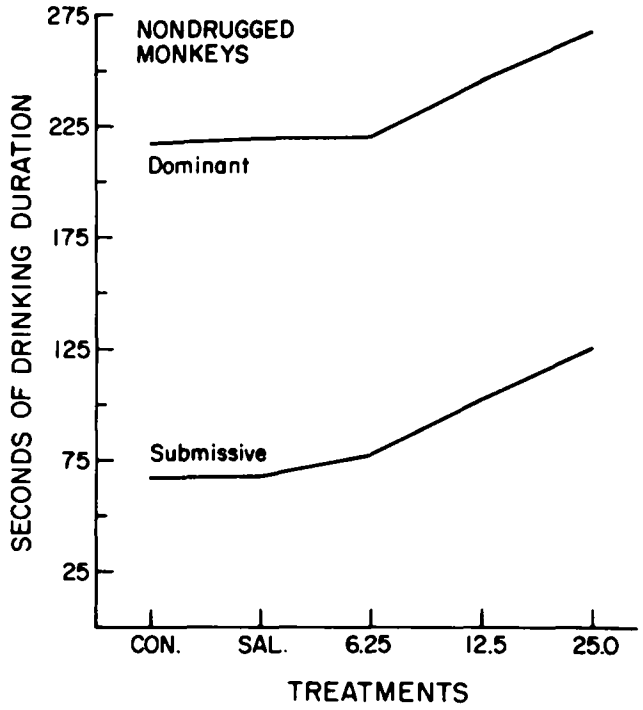

FIG. 4b. Mean number of seconds drinking duration per test session of dominant and submissive, non-drugged partners of drugged monkeys which were under control, saline, and 6.25, 12.5, and 25 μg/kg physostigmine conditions.

received the highest dose of physostigmine. This increase, however, was not statistically significant, $F(4,16)<1.0$.

Dominant monkeys, whether drugged or paired with drugged monkeys, consistantly reached the water spout faster than submissive monkeys. These differences were statistically significant for both drugged monkeys and their non-drugged counterparts, $F(1,4) = 17.80$, $p<0.025$; and $F(1,4) = 15.53$, $p<0.025$, respectively. There were, however, no significant changes in latency to the bottle of drugged and non-drugged monkeys resulting from drug injections, $F(4,16) = 1.08$, and $F(4,16)<1.0$ respectively.

Dominant monkeys, as presented in Figs. 3a and 3b also accumulated 15 sec of drinking faster than submissive monkeys. These differences were significant for both drugged monkeys and their non-drugged partners, $F(1,4) = 11.49$, $p<0.05$; and $F(1,4) = 15.53$, $p<0.025$, respectively. Although drug injections did not significantly change the time it took for drugged monkeys to accumulate 15 sec drinking, $F(1,16)<1.0$, the 15 sec latency measure for non-drugged partners of drugged monkeys did change. Under 12.5 and 25 μg/kg of physostigmine, non-drugged partners accumulated 15 sec of drinking significantly faster than their control or saline comparisons. This result was substantiated by an ANOVA and Tukey's HSD test, $F(4,16) = 7.89$, $p<0.005$; and 12.5 and 25 μg/kg<control and saline, $p<0.05$.

As indicated in Figs. 4a and 4b, dominant animals drank longer than submissive monkeys. These differences were significant regardless of whether monkeys were drugged or paired with drugged monkeys, $F(1,4) = 8.35$, $p<0.05$; and $F(1,4) = 121.45$, $p<0.001$, respectively. Also illustrated in Fig. 4a is the fact that as drug dose increased, drinking duration of dominant monkeys decreased. This result was confirmed by a significant main effect and interaction of a 2 x 5 ANOVA and post-hoc Tukey's HSD test. Drugged monkeys drank significantly less when they received 25 μg/kg physostigmine than under 6.25 μg/kg drug, saline, or control conditions, $F(4,16) = 5.23$, $p<0.01$; and 25 μg/kg<6.25 μg/kg drug, control, or saline, $p<0.01$. The diminance x treatment interaction also proved significant, $F(4,16) = 4.42$, $p<0.025$. This appears to be due to a stronger effect of drug on dominant monkeys' drinking. As presented in Fig. 4b, non-drugged monkeys appeared to take advantage of the drugged state of their partners by accumulating more drinking time than under control and saline conditions. An ANOVA and Tukey's HSD test determined that non-drugged monkeys drank reliably more when their partners received 25 or 12.5 μg/kg of physostigmine as compared to their control or saline comparisons, $F(4,16) = 6.78$, $p<0.005$; and 25 and 12.5 μg/kg>control and saline, $p<0.05$.

Finally, observers typically could not identify drugged monkeys. On six of 90 occasions when the monkeys were drugged, the observers noted that motor activity of the drugged monkeys was substantially less than normal. These observations were restricted to the two most dominant monkeys receiving the highest dose of physostigmine (25 μg/kg). On half of these occasions the number of aggressive responses recorded for drugged monkeys was substantially less than that normally recorded for the monkeys.

DISCUSSION

Increased cholinergic activity resulting from physo-

stigmine injections resulted in a general increase in aggressive responses of the squirrel monkeys observed in a water competition situation. The dose-response curve which was an inverted-U for aggressive behaviors of drugged monkeys was also typical for other behaviors investigated with physostigmine [3, 22, 23, 26] Although the effect was similar for dominant and submissive monkeys, the effect was somewhat more pronounced for dominant animals. Aggressive responding may have been suppressed in submissive monkeys when they were paired with dominant monkeys. No aggressive dominance reversals were observed during the course of the study.

As reported in previous research using rats [1,17] general activity decreased as a function of increasing cholinergic stimulation. The fact that physostigmine caused a linear decrease in general motor activity precludes the possibility that the drug-induced increase in aggression was due to a general increase in activity level of the animals. That is to say, the aggression increase could not be caused by a greater probability of the monkeys to encounter each other in the test chamber because of increased motor activity of the drugged partner.

Along with a drug-induced decrease in motor activity, a general depression was observed in one measure of drugged monkeys' competitive drinking behaviors. This decrease in drinking concurs with previous research on rats using peripheral injections of cholinergic stimulants [1, 10, 11]. Drinking duration, which correlated perfectly with aggressive dominance in pre-experimental testing, decreased as a function of drug injections. As indicated by the treatment × dominance interactions, the effect primarily resulted from the decrease in drinking duration of dominant monkeys. Since drinking duration of submissive monkeys was already low under control and saline conditions, only a small decrease in drinking duration was possible.

As reported in previous research, there was a tendency for non-drugged monkeys to react to the drugged state of their partners. Leary and Slye [14] found that when dominant monkeys were drugged with clorpromazine, their non-drugged submissive partners obtained more food rewards. In the present study, the non-drugged partners of drugged monkeys accumulated 15 sec drinking faster and drank longer than their control and saline comparisons.

It was observed that although dominant monkeys drank less when drugged, they often huddled near the available water bottle when not drinking. Under these circumstances the more submissive animals typically approached the water bottle and attempted to drink. This approach often provoked an attack by the dominant animal in an attempt to prevent the more submissive monkey from drinking. Encounters such as these partially explain the aggression increase and the water competition decrease especially in dominant monkeys.

In conclusion, the cholinergic stimulant, physostigmine, had differential effects on two commonly used and highly correlated measures of social dominance; aggressive responses and water competition behaviors. The above data support the notion that social dominance should not necessarily be considered as a unity construct, since the muscarinic cholinergic agonist, physostigmine, had differential effects on two commonly used measures of social dominance.

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