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Influence of Pharmacological Manipulation of Dopamine and Opioid Receptor Subtypes on Stereotyped Behaviour of Restricted-Fed Fowls

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KOSTAL, L. AND C. J. SAVORY. *Influence of pharmacological manipulation of dopamine and opioid receptor subtypes on stereotyped behaviour of restricted-fed fowls.* PHARMACOL BIOCHEM BEHAV 48(1) 241-252, 1994.—Effects on environmentally induced oral stereotypies (object pecking and drinker directed activity) of antagonists and agonists of dopamine and opioid receptor subtypes were examined in individually caged broiler breeder fowls subjected to chronic food restriction. Three drugs in each category were injected intravenously at three doses, and their effects compared with those of a saline control treatment. With dopamine antagonists, inhibition of both stereotypies was most marked with haloperidol (D_2), intermediate with clozapine (D_4), and lowest with SCH 23390 (D_1). Increased sitting with the high doses of these three drugs may reflect sedation. With dopamine agonists, SKF 38393 (D_1) suppressed both stereotypies slightly, quinpirole (D_3) did so consistently and potently, possibly reflecting preferential presynaptic action, while bromocriptine (D_2) inhibited drinker-directed activity consistently, but its initial suppression of object pecking changed to delayed stimulation with the high dose. This biphasic effect of bromocriptine may reflect change from pre- to postsynaptic action. Two of the opioid antagonists, naltrexone (mu) and MR 2266 (kappa, but also mu), inhibited object pecking partially, while naltrindole (delta) and the opioid agonists fentanyl (mu), BUBU (delta), and PD 117302 (kappa) had delayed and minor effects. These results suggest that expression of object pecking, but not necessarily drinker-directed activity, depends more on activation of D_2 dopamine receptors than D_1 receptors, the role of D_3 and D_4 receptors is less clear, and activation of mu and possibly kappa opioid receptors may play a contributory role.

Dopamine Opioid Receptor subtypes Antagonists Agonists Environmentally induced oral stereotypies Food restriction Fowls

THE definition of stereotypies as repetitive actions that are fixed in form and orientation with no obvious function (40,70) can be criticised because of its subjective and simplistic nature (15), and because distinction between stereotyped and nonstereotyped behaviour in some contexts may be misleading (50). Nevertheless, in pigs, cattle, and poultry, oral activities that can be broadly classed as stereotypies appear to be a consequence of restricted feeding practices that form part of commercial production of those species in certain contexts (2, 44,48,49,59).

With poultry, the commonest form of food restriction is that applied to parent stock (breeders) of meat-type chickens (broilers). The ultimate reason is that genetic selection for faster growth in progeny has been accompanied by increased appetite in the parent lines. If broiler breeders are allowed to eat as much as they want, their fertility is reduced and mortality increased. To avoid these problems, and reduce food costs, they are fed (daily in the UK) on restricted rations during rearing according to programmes recommended by the breeding companies (25,29).

Growing broiler breeders fed on the commercial rations eat only a quarter to a half as much as they would with free access to food, depending on age and on whether birds of the same age or weight are compared, and are highly motivated to eat at all times (51). They are much more active than ad lib-fed control birds, they show increased walking before feeding time

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and increased drinking and pecking at nonfood objects afterwards, and their expression of these activities is correlated with the level of food restriction imposed (31,49,50).

Environmentally induced stereotypies have been found to respond to pharmacological manipulation of both dopaminergic and opioid peptide systems. Thus, treatment with haloperidol, a dopamine receptor antagonist, suppressed oral stereotypies in pigeons and pigs, and jumping in bank voles (23, 30,65). The voles' jumping was also suppressed by alpha-methyl-para-tyrosine, a tyrosine hydroxylase inhibitor, but not fusaric acid, a dopamine beta hydroxylase inhibitor, and increased by L-DOPA (41). In ad lib-fed chickens, pigeons, and pigs, the dopamine receptor agonist apomorphine stimulated oral behaviour (8,60,71) that bore some resemblance to their stereotypies when restricted fed. Lastly, the opioid receptor antagonists naloxone, naltrexone, nalmefene, or diprenorphine suppressed environmentally induced oral stereotypies in broiler breeders, pigs, dogs and horses, and also the voles' jumping stereotypy (14,18,19,30,49,52).

There appears to have been no attempt to distinguish roles of specific receptor subtypes in environmentally induced stereotypies, however, as has been done with feeding and drinking, for example, with both dopamine (12) and opioid (34) receptors. In four experiments described here, therefore, responses of individually caged restricted-fed broiler breeders were measured after intravenous injection of selective antagonists and agonists of dopamine and opioid receptor subtypes.

The dopamine antagonists tested, SCH 23390, haloperidol, and clozapine, were chosen for their respective actions as preferential antagonists of D_1, D_2, and D_4 receptors (10,26,57, 64,67), while SKF 38393, bromocriptine, and quinpirole were chosen as agonists of D_1, D_2, and D_3 receptors (27,53,57,67). Similarly, preferential antagonists of mu, delta, and kappa opioid receptors tested were naltrexone [which is more potent and longer acting than its cogener, naloxone (24)], naltrindole and MR 2266 (35,36,42,46,72), and the respective agonists of those receptors were fentanyl, BUBU, and PD 117302 (3,11,16,32,35,36). All these compounds (including BUBU, the only peptide) are known to cross the blood-brain barrier and act centrally, but some are less selective in their actions than others (see the Discussion section).

METHOD

Animals and Husbandry

In each experiment, there were 10 immature female broiler breeder fowls (Ross 1, Ross Breeders Ltd., Midlothian, UK), that were 11 to 18 weeks old and weighed 1.09 to 1.77 kg (mean values) at the time of testing. They were tested in individual cages measuring 30 × 45 × 41 cm (W × D × H) in a three-tiered battery, where they had been housed for at least 6 weeks before testing commenced. They were fed ad lib for the first 2 weeks of life, and thereafter with weighed restricted rations provided daily at 0900 h, according to a programme recommended in the Ross 1 Parent Stock Management Manual. At the time of testing, they were receiving a grower diet (150 g/kg protein and 11.0 MJ/kg metabolisable energy) in pellet form from a food trough just outside the front of each cage, and they consumed all their daily ration in <15 min. Drinking water was available ad lib from a 1-l plastic container situated next to the feeder. Lights were on from 0600 to 2000 h, and ambient temperature was maintained at about 21°C.

Experimental Protocol

The four experiments were done two at a time, dopamine [1] and opioid [3] antagonists together followed by dopamine [2] and opioid [4] agonists. Birds in one experiment were in alternate cages in the battery, separated by birds in the other experiment. Each experiment lasted 5 weeks, one being conducted on Mondays and Thursdays and the other on Tuesdays and Fridays; birds were weighed on Wednesdays. Within each experiment, the 10 birds received 10 injection treatments (three drugs at three doses and a 0.9% saline control), each bird receiving a different treatment on each day according to a Latin square arrangement. Low, medium, and high doses of each drug were in the proportions 1 : 5 : 25 and were based on results of previously published research and pilot trials. All birds were injected by wing vein with 1 ml/kg between 1010 and 1025 h (i.e., 1 h after feeding time), and their behaviour was recorded on videotape for 3 h after the last injection.

Measurements were made from the 3 h videorecordings in six alternate 15 min time periods, commencing at the start, by noting each injected bird's behaviour in every minute from a single "on the dot" observation (56), according to one of six categories. These were sitting (only), standing (only), pacing, preening (mainly while standing, occasionally while sitting), object pecking (at the empty feeder or parts of the cage), or drinker-directed activity [drinking was interspersed with, and indistinguishable from, pecking at the water or drinker without drinking; most birds produced wet faecal droppings, indicating polydipsia (33)]. The last two activities (but not pacing or preening) were stereotyped in form, according to the defini-

TABLE 1

SIGNIFICANCE OF EFFECTS OF BIRD, INJECTION DAY, INJECTION TREATMENT, AND CARRYOVER FROM THE PRECEDING TREATMENT, IN SIX TIME PERIODS AND SIX ACTIVITIES, IN EXPERIMENT 1 (DOPAMINE ANTAGONISTS)

Effect	Bird						Day						Treatment						Carryover					
Time Period*	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Activity																								
Sitting	c	a	–	–	–	a	a	c	b	b	–	c	c	c	b	c	c	c	–	–	–	–	–	–
Standing	c	c	c	c	c	c	–	a	–	c	–	–	b	c	a	b	c	–	–	–	–	–	–	–
Pacing	–	a	a	c	c	b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	a	–	–
Preening	–	–	–	–	b	–	–	–	–	–	a	–	–	–	–	–	a	–	–	–	–	–	–	–
Object pecking	c	c	c	c	c	c	–	–	–	a	a	a	c	c	c	c	c	c	–	–	–	–	–	–
Drinker directed	a	a	b	b	–	a	–	–	a	–	–	–	c	–	b	a	b	a	–	–	a	–	–	–

*Time periods refer to alternate 15 min in the 3 h after injections ended (see the Method section).
–Not significant ($p > 0.05$); [a]$p < 0.05$; [b]$p < 0.01$; [c]$p < 0.001$.

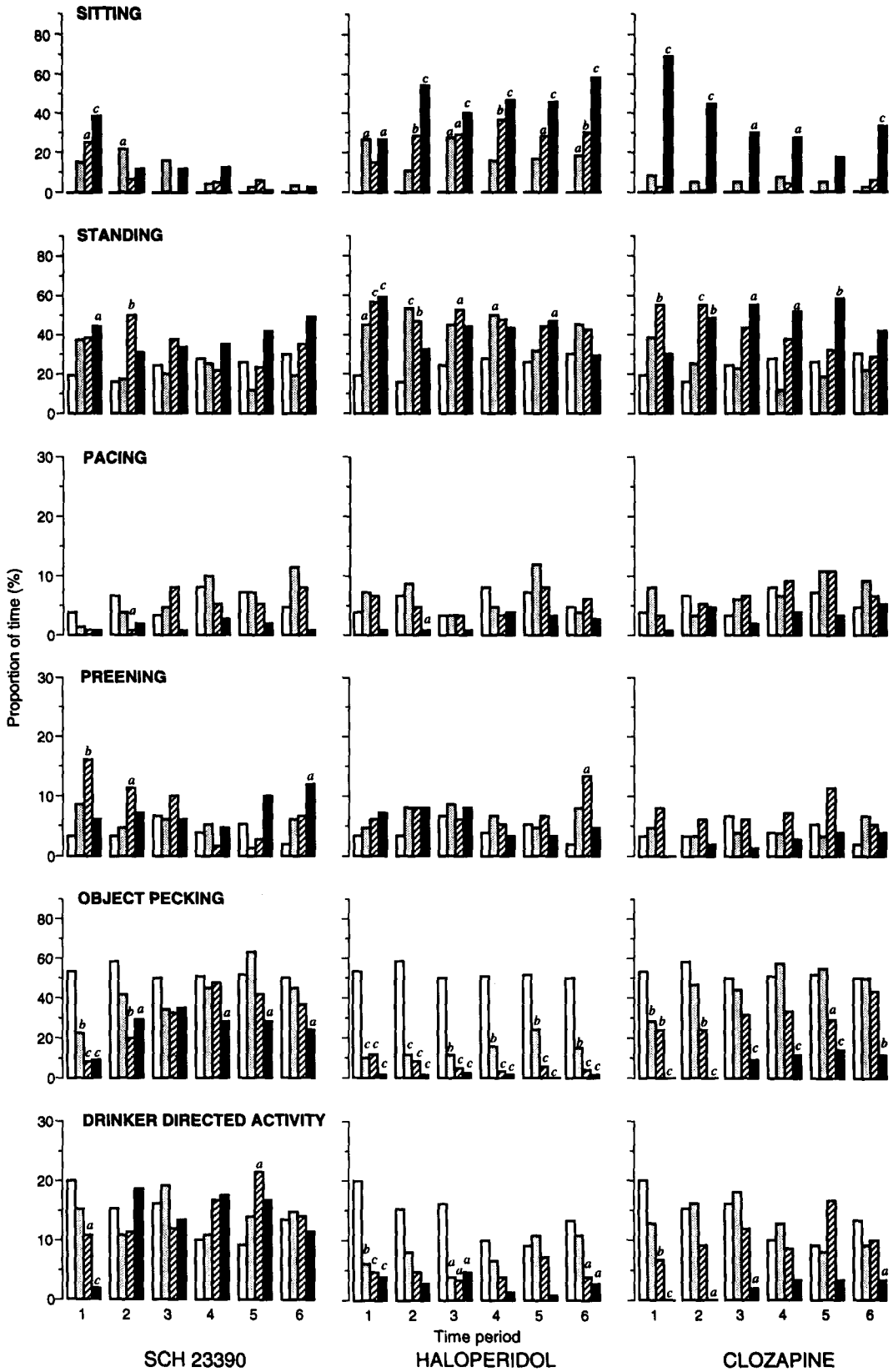

FIG. 1. Mean (n = 10) proportions of time spent per bird in different activities in six alternate 15 min periods, after intravenous injection (1 ml/kg) of either saline (□) or low (▨), medium (▨), or high (■) doses of three dopamine receptor antagonists (see the Method section for actual doses) in Experiment 1. Superscripts above columns represent significant differences from the saline treatment in that time period; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

tion in the introduction section (40,70). From these observations were calculated proportions of time spent by each bird in each activity in each time period. The computer software used for this analysis was written by LK in Turbo Pascal (Borland International, Scotts Valley, CA).

In the Latin square experimental design, each of the 10 injection treatments was followed by each of the other treatments once on succeeding injection days, across all birds, thus balancing any carryover effects of preceding treatments (28). Birds were injected in a different random order each day, so that, across all days, treatments were distributed evenly with respect to the time after injection (mean 8 min) when videorecording began. With each of the six activities and six time periods, a separate three-way ANOVA was carried out to determine the significance of effects of bird, injection day, injection treatment, and carryover from the preceding treatment. In addition, within each time period, each drug treatment was compared with the saline control treatment by *t*-test. No statistical comparisons between drugs were made because equivalent doses (low, medium, high) of different drugs were not equimolar.

Experiment 1, Dopamine Antagonists

SCH 23390 hydrochloride [D_1, Research Biochemicals Incorporated (RBI), Natick, MA] was dissolved in 0.9% saline and doses injected were 0.04, 0.2, and 1.0 mg/kg. Haloperidol (D_2, RBI) was dissolved in 1 M acetic acid and doses were 0.04, 0.2, and 1.0 mg/kg. Clozapine (D_4, Sandoz Pharmaceuticals, Horsforth, UK) was dissolved in 0.1 M hydrochloric acid and doses were 0.4, 2.0, and 10.0 mg/kg. Acidic solutions were adjusted to pH 5.5–6.5 by titration with 1 M sodium hydroxide, and diluted to required concentrations with distilled water.

Experiment 2, Dopamine Agonists

SKF 38393 hydrochloride (D_1, RBI) was dissolved in distilled water and doses were 0.64, 3.2, and 16.0 mg/kg. Bromocriptine methanesulfonate (D_2, RBI) was dissolved in 1.5% tartaric acid in absolute alcohol, diluted to required concentrations with saline, and doses were 0.32, 1.6, and 8.0 mg/kg. Quinpirole hydrochloride (D_3, RBI) was dissolved in saline and doses were 0.16, 0.8, and 4.0 mg/kg.

Experiment 3, Opioid Antagonists

Naltrexone hydrochloride (mu, RBI) was dissolved in saline and doses were 0.4, 2.0, and 10.0 mg/kg. Naltrindole hydrochloride (delta, RBI) was dissolved in 0.1 M hydrochloric acid and doses were 0.08, 0.4, and 2.0 mg/kg. MR 2266 (kappa, Boehringer, Ingelheim, Germany) was dissolved in 0.1 M hydrochloric acid and doses were 0.08, 0.4, and 2.0 mg/kg. Acidic solutions were adjusted to pH 5.5–6.5 and diluted to required concentrations with distilled water.

Experiment 4, Opioid Agonists

Fentanyl citrate (mu, RBI) was dissolved in saline and doses were 3.2, 16.0, and 80.0 μg/kg. BUBU (delta, Neosystem Laboratoire, Strasbourg, France) was dissolved in saline and doses were 0.064, 0.32, and 1.6 mg/kg. PD 117302 (kappa, Parke-Davis/Warner-Lambert, Ann Arbor, MI) was dissolved in distilled water and doses were 0.064, 0.32, and 1.6 mg/kg.

RESULTS

Experiment 1, Dopamine Antagonists

Of the 36 ANOVAs with data from Experiment 1 (six activities and six time periods), there were significant ($p < 0.05$) effects of bird in 26, of injection day in 12, of injection treatment in 23, and of carryover from the preceding treatment in two (Table 1).

With the saline control treatment, overall mean proportions of time spent in different activities were 0% sitting, 23.8% standing, 5.7% pacing, 4.1% preening, 52.5% object pecking, and 14.0% drinker-directed activity. When compared with saline, the three dopamine antagonists had similar effects on behaviour. Thus, SCH 23390 (D_1), haloperidol (D_2), and clozapine (D_4) all tended to inhibit the oral stereotypies (object pecking and drinker-directed activity), and to increase sitting and standing (Fig. 1). SCH 23390 and haloperidol also reduced pacing and increased preening. In most cases, these effects commenced rapidly and their duration was related to the dose injected, but in a few, the effects were sporadic or delayed. In terms of total numbers of significant differences when compared with saline, across all time periods, inhibitory effects on oral stereotypies were most marked with haloperidol, intermediate with clozapine and lowest with SCH 23390.

Experiment 2, Dopamine Agonists

Of the 36 ANOVAs in Experiment 2, there were significant effects of bird in 16, of day in 1, of treatment in 18, and of carryover in 2 (Table 2).

TABLE 2

SIGNIFICANCE OF EFFECTS OF BIRD, INJECTION DAY, INJECTION TREATMENT, AND CARRYOVER FROM THE PRECEDING TREATMENT, IN SIX TIME PERIODS AND SIX ACTIVITIES, IN EXPERIMENT 2 (DOPAMINE AGONISTS)

Effect	Bird						Day						Treatment						Carryover					
Time Period	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Activity																								
Sitting	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Standing	b	–	–	b	a	a	–	–	–	–	–	–	c	c	c	c	c	c	–	a	–	–	–	–
Pacing	b	–	a	–	–	b	–	–	–	–	–	–	–	–	–	–	–	–	–	a	–	–	–	–
Preening	–	–	–	b	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Object pecking	–	a	–	–	–	c	–	–	–	–	–	–	c	b	a	a	c	c	–	–	–	–	–	–
Drinker directed	b	b	b	b	a	b	a	–	–	–	–	–	c	c	c	c	b	c	–	–	–	–	–	–

Details as for Table 1.

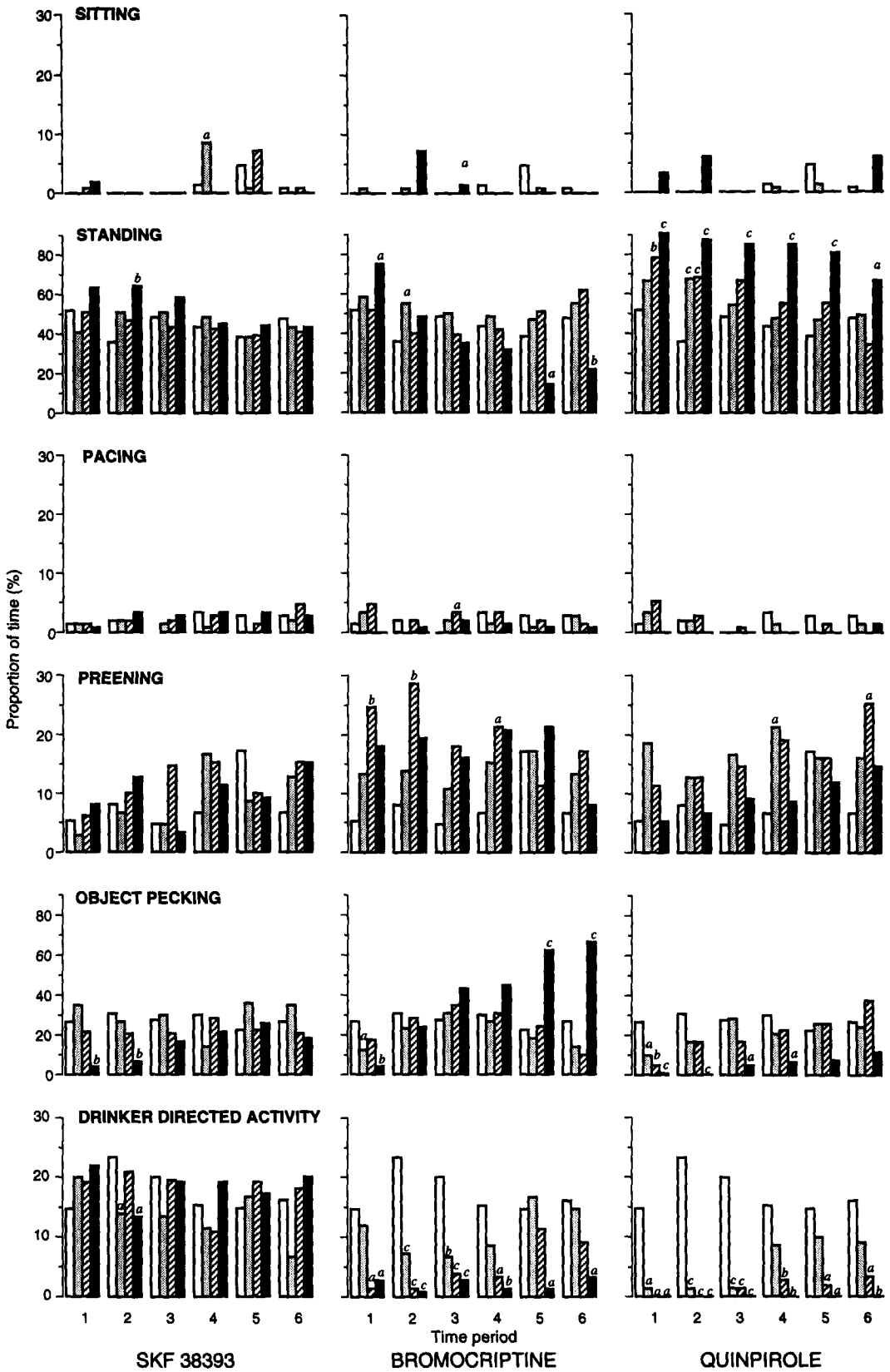

FIG. 2. Mean (n = 10) proportions of time spent per bird in different activities in six alternate 15 min periods, after intravenous injection (1 ml/kg) of either saline (□) or low (▨), medium (▨), or high (■) doses of three dopamine receptor agonists (see the Method section for actual doses) in Experiment 2. Superscripts above columns represent significant differences from the saline treatment in that time period; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

With saline, overall mean proportions of time spent in different activities were 1.1% sitting, 44.1% standing, 2.0% pacing, 8.1% preening, 27.4% object pecking, and 17.3% drinker directed. After injection, all three dopamine agonists, SKF 38393 (D_1), bromocriptine (D_2), and quinpirole (D_3), suppressed object pecking and drinker-directed activity, and increased standing, in a dose-related way (Fig. 2). With the high dose of bromocriptine, however, there was delayed stimulation of object pecking and inhibition of standing. In terms of numbers of significant differences from saline, effects on oral stereotypies of bromocriptine and quinpirole were much greater than those of SKF 38393.

Experiment 3, Opioid Antagonists

Of the 36 ANOVAs in Experiment 3, there were significant effects of bird in 24, of day in four, of treatment in five, and of carryover in one (Table 3).

With saline, overall mean proportions of time spent in different activities were 0.2% sitting, 13.0% standing, 3.5% pacing, 3.9% preening, 69.4% object pecking, and 10.1% drinker directed. After injection, object pecking was suppressed by the high dose of naltrexone (mu receptor antagonist) in the first three time periods, and the high dose of MR 2266 (kappa) in the first period (Fig. 3). There were concomitant increases in standing and pacing with naltrexone, and in sitting, standing, and preening with MR 2266. Subsequent inhibition of object pecking by low doses of MR 2266 and naltrindole (delta), and of drinker-directed activity by naltrindole, was less significant. Compared with the dopaminergic compounds in Experiments 1 and 2, effects of the opioid antagonists on oral stereotypies were weak.

Experiment 4, Opioid Agonists

Of the 36 ANOVAs in Experiment 4, there were significant affects of bird in 27, of day in seven, of treatment in three, and of carryover in none (Table 4).

With saline, overall mean proportions of time spent in different activities were 0.8% sitting, 45.8% standing, 0.7% pacing, 12.9% preening, 27.2% object pecking, and 12.7% drinker directed. The three opioid agonists, fentanyl (mu), BUBU (delta), and PD 117302 (kappa), had least effect on behaviour when compared with the compounds in Experiments 1, 2, and 3. There was a delayed increase in object pecking with the high dose of BUBU, and delayed suppression of drinker-directed activity with the low dose of fentanyl and high dose of PD 117302 (Fig. 4). There were also increases in sitting, pacing, and preening with BUBU, in sitting and pacing with PD 117302, and a decrease in standing with PD 117302.

DISCUSSION

The purpose of these experiments was to identify dopamine and opioid receptor subtypes that are implicated closest with the oral stereotypies of caged restricted-fed broiler breeders, i.e., object pecking and drinker-directed activity. Superficially, in terms of numbers of significant effects on these activities, across all experiments, treatments, and time periods, the dopaminergic compounds were much more potent (93 differences compared with the saline control treatment) than the opioid compounds (11 differences), within the range of doses tested. Furthermore, the results of Experiments 1 and 2 indicate that the stereotypies were influenced more by manipulation of D_2, D_3, and D_4 receptors (with haloperidol, clozapine, bromocriptine, and quinpirole) than by manipulation of D_1 receptors (with SCH 23390 and SKF 38393). Similarly, the results of Experiment 3 indicate that blockade of mu and kappa opioid receptors inhibited object pecking, while delta receptor blockade and the opioid agonist treatments in Experiment 4 had relatively little effect. These impressions, however, need to be examined carefully.

First, there is the question of whether both stereotypies responded equally to the various treatments. In terms of number of significant effects, object pecking responded more than drinker-directed activity to treatment with all three dopamine antagonists (Fig. 1). With the dopamine agonists, SKF 38393 acted more on object pecking, while bromocriptine and quinpirole acted more on drinker-directed activity (Fig. 2). The high dose of bromocriptine, however, had a biphasic effect on object pecking only, eventual stimulation following initial suppression. Also, two of the opioid antagonists, naltrexone and MR 2266, inhibited object pecking only (Fig. 3). Such differences between stereotypies might reflect variation in their underlying control, but they might also reflect the fact that object pecking was always the dominant stereotypy with saline.

Second, there is the question of whether observed inhibition of stereotypies was a specific response, or part of more general inhibition of motor activity, perhaps associated with sedation. This is not easy to assess, especially because inhibition of stereotypies, as measured in proportions of time, must inevitably be reflected by increases elsewhere. There is little

TABLE 3

SIGNIFICANCE OF EFFECTS OF BIRD, INJECTION DAY, INJECTION TREATMENT, AND CARRYOVER FROM THE PRECEDING TREATMENT, IN SIX TIME PERIODS AND SIX ACTIVITIES, IN EXPERIMENT 3 (OPIOID ANTAGONISTS)

Effect	Bird						Day						Treatment						Carryover					
Time Period	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Activity																								
Sitting	–	–	–	–	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	a	–
Standing	–	a	c	c	c	c	–	–	–	–	a	b	a	–	–	–	–	–	–	–	–	–	–	–
Pacing	c	c	b	c	c	c	–	–	–	–	–	–	–	–	b	–	–	–	–	–	–	–	–	–
Preening	–	b	–	–	–	–	–	–	–	–	–	a	c	–	–	–	–	–	–	–	–	–	–	–
Object packing	b	c	c	c	c	c	–	–	–	–	–	–	b	–	–	–	–	–	–	–	–	–	–	–
Drinker directed	c	b	b	c	a	–	–	–	a	–	–	–	a	–	–	–	–	–	–	–	–	–	–	–

Details as for Table 1.

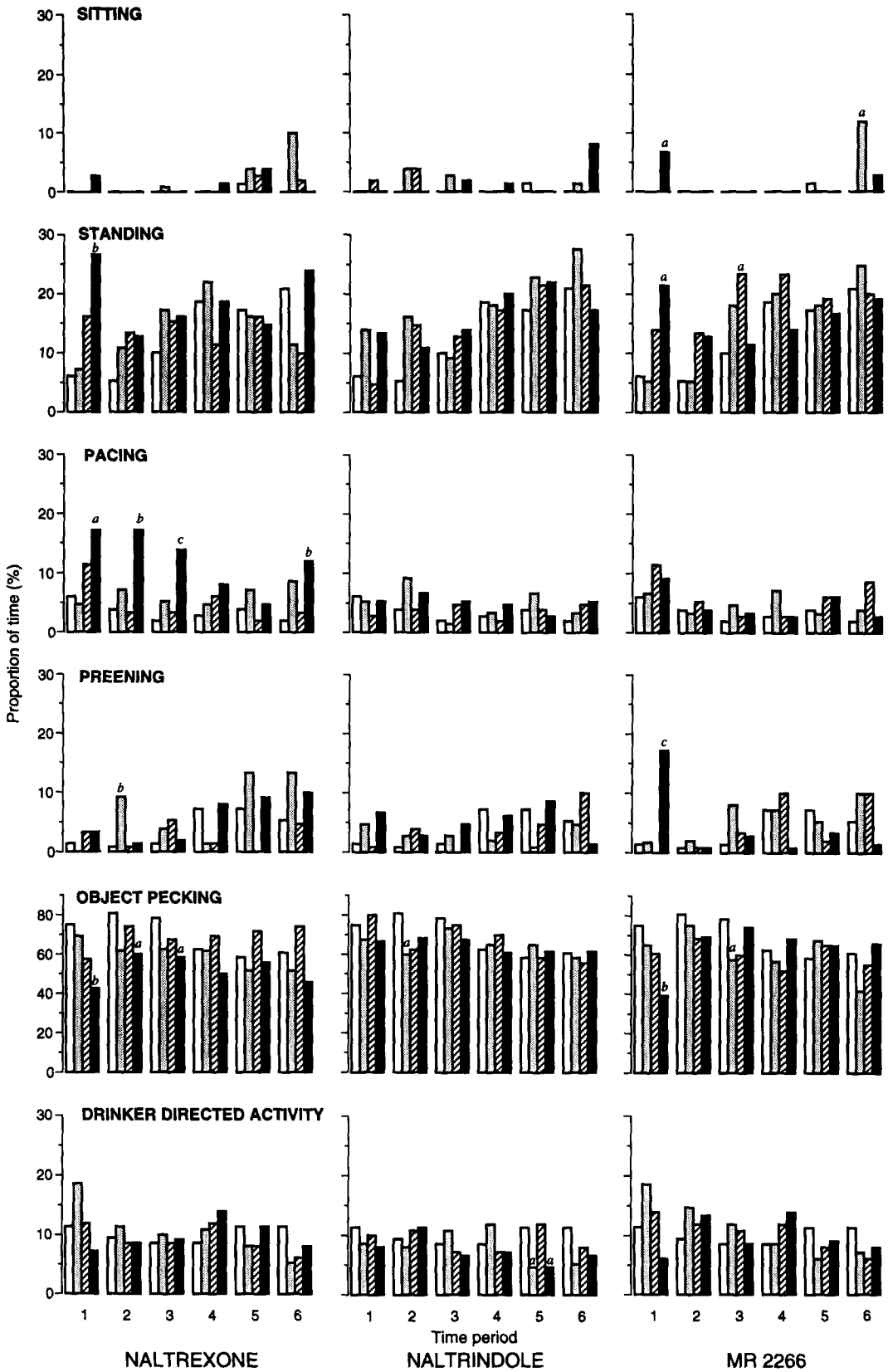

FIG. 3. Mean (n = 10) proportions of time spent per bird in different activities in six alternate 15 min periods, after intravenous injection (1 ml/kg) of either saline (□) or low (▨), medium (▨), or high (■) doses of three opioid receptor antagonists (see the Method section for actual doses) in Experiment 3. Superscripts above columns represent significant differences from the saline treatment in that time period; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

TABLE 4

SIGNIFICANCE OF EFFECTS OF BIRD, INJECTION DAY, INJECTION TREATMENT, AND CARRYOVER FROM THE PRECEDING TREATMENT, IN SIX TIME PERIODS AND SIX ACTIVITIES, IN EXPERIMENT 4 (OPIOID AGONISTS)

Effect	Bird						Day						Treatment						Carryover					
Time Period	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Activity																								
Sitting	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Standing	c	c	c	c	c	c	–	a	c	–	a	–	a	–	–	–	–	–	–	–	–	–	–	–
Pacing	a	a	b	–	–	–	b	–	–	–	a	–	a	–	–	a	–	–	–	–	–	–	–	–
Preening	c	c	c	c	c	c	–	–	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Object pecking	c	c	c	c	c	c	–	–	a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Drinker directed	c	c	c	a	b	c	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Details as for Table 1.

evidence in any of the experiments to suggest general inhibition of motor activity, because there was no occasion when significant inhibition of both stereotypies coincided with significant inhibition of both other obvious forms of motor activity, pacing and preening (some movement also occurred during sitting and standing). No attempt was made to distinguish alertness (e.g., head up vs. head down) during sitting and standing, but it seems likely that the increased sitting seen in Experiment 1 with medium and/or high doses of all three dopamine antagonists (Fig. 1) could reflect some sedation. This effect was confined to the first 15 min with SCH 23390, but continued throughout the 3 h observation period with haloperidol and clozapine. In the other experiments there was increased sitting and standing with the high dose of MR 2266 (Fig. 3) in the first 15 min, but this coincided with increased preening, and there appeared to be no sedation associated with inhibition of object pecking with the high dose of naltrexone, because it coincided with increased pacing.

Third, there is the question of how specific compounds were in their actions on particular receptors. SCH 23390 is a potent and selective D_1 receptor antagonist, but has marginal effects on D_2, alpha1-adrenergic, muscarinic, histaminergic, 5-HT_{1C} and 5-HT_2 receptors (26,69). Haloperidol lacks affinity for D_1 (9), and has much greater affinity for D_2 than D_3 (57) or D_4 (64) receptors, but acts residually on alpha-adrenergic, glutamate (4) and sigma receptors (68). Clozapine's affinity for D_4 is much greater than for related D_2 and D_3 receptors (64), but it interacts potently with alpha-adrenergic (43) and 5-HT_2 (37) receptors. In Experiment 1, therefore, there would appear to be little overlap between drugs in their actions on different dopamine receptors, but effects of their interactions with other neurotransmitter systems cannot be excluded.

SKF 38393 was, until recently, the only agonist with selective action on D_1 receptors (55,67), and has no known residual properties. Bromocriptine is a slightly more potent agonist of D_2 than D_3 receptors (57), and has weak effects at D_1, D_4, alpha-adrenergic, 5-HT_1, and 5-HT_2 receptors (27,64,67). Quinpirole's agonist action is much greater at D_3 than D_2 receptors in the rat (57), but not in canine striatum, where its D_2 action is dominant over D_3, and where its affinity for other receptors is negligible (53). Relative affinities of bromocriptine and quinpirole for D_2 and D_3 receptors in chicken brain are not known, and there may have been overlap in their actions in Experiment 2.

Naltrexone is a more potent and longer-acting opioid antagonist than its *N*-allyl cogener, naloxone (24), and while both bind to mu, delta, and kappa receptors, they have highest affinity for mu (35,36). Naltrindole is currently the most selective antagonist of the delta receptor, with negligible affinity for mu and kappa sites (35,42). MR 2266 has been reported as a selective kappa antagonist (35), but its potency at the kappa receptor is, in fact, only twice as great as at mu, and nine times as great as at delta (36). Hence, there could have been overlap in central actions of naltrexone and MR 2266 that could account for their similar effect on object pecking in Experiment 3.

Fentanyl, BUBU [a peptide that crosses the blood–brain barrier, (16)] and PD 117302 have all been shown to be highly selective and potent ligands at mu, delta, and kappa receptors, respectively (11,22,35,36). There should, therefore, have been little overlap in their effects in Experiment 4.

Fourth, there is the question of why inhibition of stereotypies occurred with dopamine agonist as well as antagonist treatments. With the antagonists, this inhibition presumably reflects suppression of dopaminergic neurotransmission, while with the agonists, there are differences in the way in which they act pre- and postsynaptically, which can have opposite effects on behaviour. D_1 receptors are thought to be located only postsynaptically (10), and SKF 38393, the D_1 agonist, has consistently failed to stimulate stereotyped behaviour, here (Fig. 2) and in previous studies with rats and chicks (54,66,71). It is not known why SKF 38393 acts in this way, when the D_1 antagonist SCH 23390 also suppressed both stereotypies here (Fig. 1), as well as schedule-induced polydipsia and drug-induced stereotypies in rats and chicks (62,67,71).

D_2 and D_3 receptors are located pre- and postsynaptically (10,57), and the proposed role of these presynaptic receptors (autoreceptors) is to self-regulate synthesis and release of dopamine (7). D_2 and D_3 agonists act at presynaptic receptors to inhibit dopaminergic neurotransmission, and this inhibition is greater with systemic than with local injection because the former affects somatodendritic as well as terminal autoreceptors (1,61). Both bromocriptine and quinpirole produce dose-dependent biphasic effects on locomotor activity in rodents. Low doses cause inhibition only, due to presynaptic action, while high doses cause inhibition followed by delayed stimulation due to postsynaptic action (20,27,38). This mechanism can presumably account for the delayed stimulation of object pecking here with the high dose of bromocriptine (Fig. 2). The fact that quinpirole did not do likewise could be because its high dose was not sufficient to produce such an effect, or

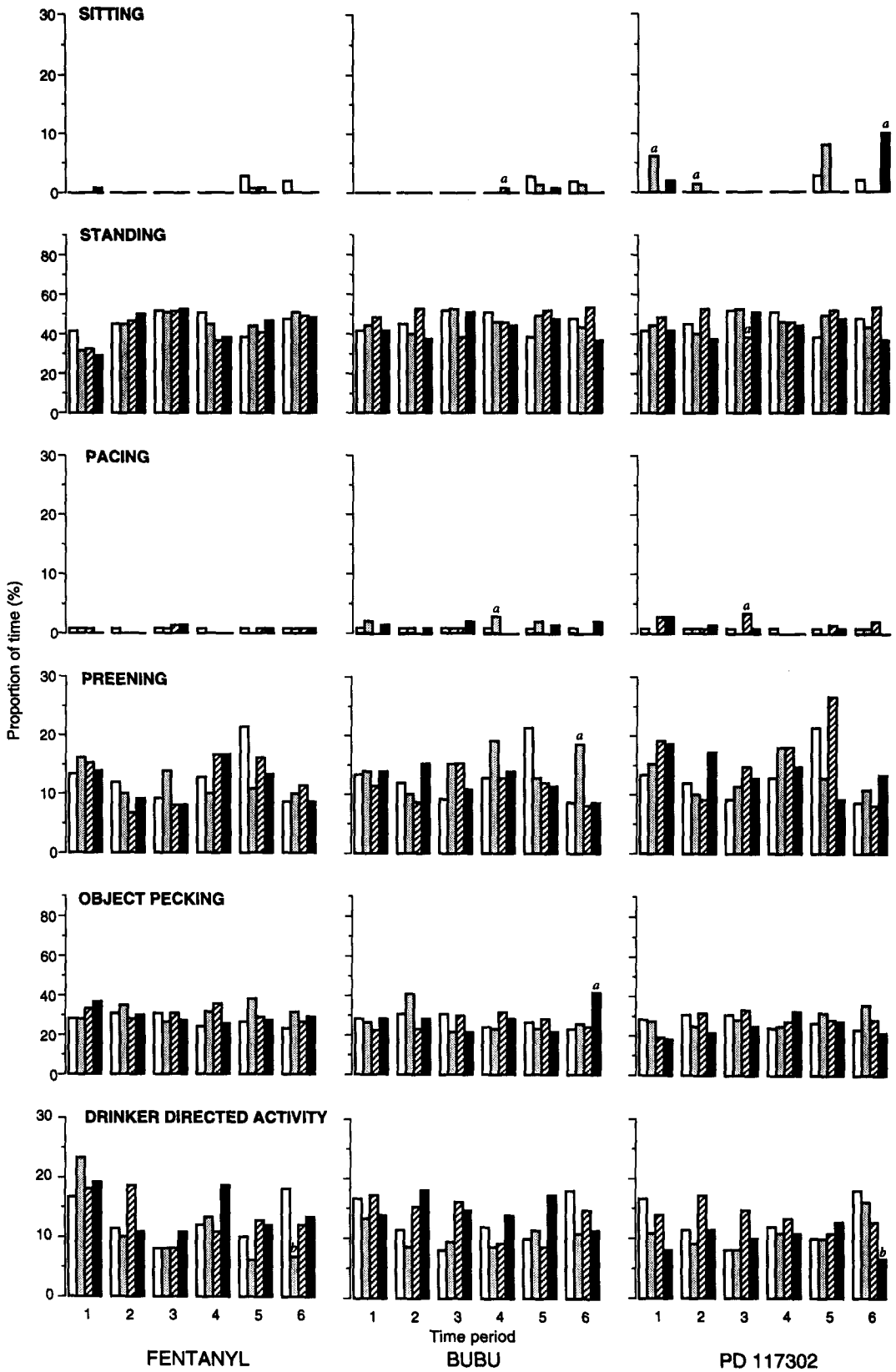

FIG. 4. Mean (n = 10) proportions of time spent per bird in different activities in six alternate 15 min periods, after intravenous injection (1 ml/kg) of either saline (□) or low (▨), medium (▨), or high (■) doses of three opioid receptor agonists (see the Method section for actual doses) in Experiment 4. Superscripts above columns represent significant differences from the saline treatment in that time period; $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

because it may be a more selective autoreceptor agent than bromocriptine (57). Moreover, a difference in the chicken's behavioural response, including pecking, to two D_2 agonists, CQ 32-084 and CQP 201-403, was also interpreted in terms of different affinities towards pre- and postsynaptic receptors (21). Hence, object pecking is not necessarily implicated closer with D_2 than with D_3 receptors. Both bromocriptine and quinpirole have been reported to interact synergistically with D_1 agonists in stimulating motor activities (27,45,71).

The inhibitory effect of haloperidol here (Fig. 1) is like that reported with environmentally induced stereotypies (including schedule-induced polydipsia) in other species (23,30,62,65), while D_2 antagonists in general are also potent inhibitors of drug-induced stereotypies (67,71). Although haloperidol and clozapine acted similarly here, their effects on selective components of drug-induced behaviour have been shown to differ (63).

Fifth, there is the question of why the opioid agonists failed to stimulate stereotypies, when two of the antagonists, naltrexone and MR 2266, suppressed object pecking (Figs. 3 and 4), and nalmefene, an analogue of naltrexone, did likewise in a previous experiment (49). This effect of mu and kappa antagonists concurs with the finding that both mu and kappa (but not delta) agonist treatments stimulate oral stereotypies in rats (39,47). However, because naltrexone has higher affinity for the mu receptor, while MR 2266 has more equal potency at both mu and kappa sites (36), it is possible that their common effect on pecking could reflect action at the mu receptor only. Also, although effects of the agonist treatments were not significant here, there were tendencies for object pecking to increase with fentanyl and decrease with PD 117302 in the first 15 min; there was no such effect with BUBU (Fig. 4). These tendencies could reflect the opposite effects that mu and kappa agonists are reported to have on dopamine release, increasing and decreasing it, respectively (17). Behavioural responses to manipulation of opioid receptors could thus be mediated at least partly through a dopaminergic mechanism [see (13) for a review on opioid and dopamine interactions].

Sixth, there is the question of whether birds' responses were influenced by the fact that they each received 10 treatments in the experimental design. For example, repeated injections of SKF 38393 or quinpirole caused changes in D_1 and D_2 receptor densities, respectively, in rats (58), and also corresponding changes in behaviour, including stereotypies (5). In all the experiments here, however, significant (carryover) effects of previous treatment were negligible (Tables 1, 2, 3, and 4). On the other hand, significant bird effects (individual variation) were frequent (Tables 1, 2, 3, and 4), but their influence on results should have been balanced by the Latin square design.

In conclusion, these results show that object pecking and drinker-directed activity were more responsive to manipulation of D_2, D_3 and D_4 receptors, which have similar pharmacological profiles (57,64), than to manipulation of the D_1 receptor. Compared with dopamine receptors, manipulation of opioid receptors had little effect, although object pecking was suppressed partially with high doses of the antagonists naltrexone and MR 2266, possibly due to common action at the mu receptor. The results suggest that chronic food restriction, which appears to underlie expression of these oral stereotypies (50), may do so primarily through alteration of dopaminergic neurotransmission [e.g., (6)], and that concomitant variation in opioid activity may play a contributory role.

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