Social interactions in cats: regional brain monoamine distribution in dominant and submissive cats

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Abstract. Regional brain concentration of monoamines (NA, DA and 5-HT) and their metabolites (MHPG, DOPAC, HVA and 5-HIAA) were measured in dominant and submissive cats in a predatory competition test and in predatory behaviour of single cats. A submissive position in predatory hierarchy produced an increase in concentration of NA in the hypothalamus, a decrease of DA, 5-HT, MHPG and MHPG/NA ratio in the hippocampus and a decrease of 5-HT in the prefrontal cortex in comparison with dominant cats. In the predatory test the social situation increased the concentration of DA and its metabolites in the hypothalamus of the submissive and dominant cats as compared to single cats. Additionally, a diminution of NA and MHPG concentration in the midbrain in dominant cats compared to single animals in the predatory situation was observed. The data obtained demonstrate a considerable differentiation in the effects of predatory aggression and predatory behaviour in a social situation on brain distribution of monoamines. The results indicate that predatory behaviour in single and paired cats is regulated by different catecholamine mechanisms.

Key words: social interactions, predatory test, dominance and submission, brain monoamines, HPLC, cat
INTRODUCTION

For many years increasing attention has been given to the relationship between stress, social interactions and central neurochemical changes (for review see Weiss et al. 1981). The participation of monoaminergic systems in regulation of various types of emotional-defensive reactions, i.e. aggression, predatory behaviour or anxiety, has been investigated mainly in pharmacological studies (for review see Siegel and Pott 1988).

The pharmacological investigations carried out by our laboratory and by others showed that the inhibition of 5-HT synthesis following administration of p-chlorophenylalanine (PCPA) or 5,6-dihydroxytryptamine induced an increase of several types of aggression (Palermo-Neto and Carlini 1972, Sheard and Davis 1976, Valzelli et al. 1981, Romaniuk et al. 1987, 1989, 1990). Numerous studies indicated that serotonin also modulates predatory attack in cats (for review see Siegel and Pott 1988). Predatory behaviour, which is classified as one type of aggression (Moyer 1968), is facilitated after subcutaneous PCPA administration (Ferguson et al. 1970). In other studies, the stimulation of the raphe nuclei in cats exerted an inhibition of predatory behaviour (Shaikh et al. 1984). On the other hand, the results obtained from social behaviour studies employing food-competition situations to elicit aggressive behaviour revealed that the diminution of 5-HT release by a low dose of D-lysergic acid diethylamide decreased dominant behaviour in rats (Uyeno 1966).

The results concerning a role of NA in the control of predatory aggression of single animals or in social situations are contradictory. The most pronounced changes in predatory behaviour in cats were exerted by imipramine in animals with electrical brain stimulation (Dubinsky and Goldberg 1971) as well as in cats in semi-natural condition (Zagrodzka et al. 1987). Imipramine, a tricyclic antidepressant, acting mainly by inhibition of NA uptake (Lemberger et al. 1985) elicited predatory behaviour in spontaneous "non-killer" cats (Zagrodzka et al. 1987) and predatory competition leading to predatory domination by submissive cats (Zagrodzka et al. 1985). However, repeatedly administered imipramine blocked a predatory attack elicited by the hypothalamic stimulation in cats (Dubinsky and Goldberg 1971). Additionally, the elevated level of the brain catecholamines following administration of DOPA or DA potentiated aggressive behaviour in rats (Palermo-Neto and Carlini 1972, Datla et al. 1992). Dopamine facilitated aggressive behaviour in rats and cats (Shaikh et al. 1991, Dalta et al. 1992). In the studies of social behaviour administration of DOPA increased winning in the competitive situation (Masur et al. 1974, Winberg and Nilsson 1992).

The neurochemical study of monoamine concentration produced by predatory behaviour of single cats or in a social situation is a fundamental supplement to pharmacological investigations. At present, interactions between various neurotransmitter systems have been thought to be involved in the regulation of numerous types of reactions, including emotional-defensive reactions (Reader and Jasper 1984).

Therefore, in the present study concentrations of NA, DA and 5-HT and of their main metabolites MHPG, DOPAC, HVA, and 5-HIAA were measured in the anterior and posterior hypothalamus, amygdala, midbrain central gray matter, hippocampus and in the prefrontal cortex of cats engaged in predatory and social behaviour in semi-natural conditions.

The animals have been studied in two kinds of tests:
1. Predatory test - investigated the effects of predatory behaviour of single cats.
2. Predatory competition test - investigated the effects of social interactions between paired cats.

METHODS

Subjects

The experiments were performed on sixteen adult cats of either sex, weighing from 2.5 to 3.6 kg at the beginning of the study. The animals were housed in individual wire mesh cages and main-
tained in a temperature-controlled room (22±2°C) on a 12-h light-dark cycle; food (cereal with meat and milk) and tap water were available continuously.

All cats were pre-tested on their predatory abilities; each animal was a good mouse-killer, i.e., killing latency was less than 5 s during 3 consecutive sessions.

**Experimental procedure**

**EXPERIMENTAL GROUPS**

The experimental animals were used in two kinds of predatory tests, i.e., predatory test of a single animal (Single cats; n=6) or predatory test in a competitive situation for two animals: dominant cat (Dominants; n=5) and submissive cat (Submissives; n=5). The tests were performed between 10.00-12.00 a.m., in an experimental chamber (180 x 180 x 120 cm) in which the animals were able to move freely, jump and catch a mouse.

**PREDATORY TEST FOR A SINGLE ANIMAL**

The animal, after 24-h food deprivation, was placed in the experimental chamber for 3-6 min. After this time a freely moving white mouse (body wt. 25-30 g) was dropped into the chamber through a port in the upper wall. Three such tests were performed during one session i.e., three mice were dropped, one at a time, after consumption of the previous mouse. Each cat participated in twelve sessions.

**PREDATORY TEST IN A COMPETITIVE SITUATION FOR PAIRED ANIMALS**

After 24-h food deprivation, two animals of the same sex, approximate weight and killing latency were introduced at the same time into the experimental chamber for 3-6 min, and then a freely moving white mouse was dropped into the chamber. The interpartner relationship of the cats was observed. Only the pairs with marked dominance of one of the paired cats (Fonberg et al. 1985) were used in the experiment. In these pairs the established dominance was stable during three successive sessions, i.e., the dominant cat always caught, killed and ate each mouse. Twelve experimental sessions consisting of three tests each were carried out after establishing the dominance in all pairs used, in order to consolidate the established hierarchy.

**DETERMINATION OF MONOAMINES BY HPLC WITH ELECTROCHEMICAL DETECTION**

**Sample preparation**

The animals were killed by decapitation just after the end of the second test during the 12th session, between 10.00-11.00 a.m. The dominant cats were decapitated before the submissive ones. The brains were quickly removed and selected regions, i.e., the anterior hypothalamus (HA), posterior hypothalamus (HP), midbrain central gray matter (GC), hippocampus (HI), amygdala (AM) and the prefrontal cortex (CTX) were separated and kept frozen at -70°C until analysis (for details see Romaniuk et al. 1989). The selected tissues were weighed and homogenized with an ultrasonic cell disruptor (Vibracell 72434, 50 W, Bioblock) in 1 ml 0.1 M perchloric acid containing 0.4 mM sodium metabisulphite. The samples were then centrifuged at 10,000 x g for 25 min at 4°C. The supernatants were filtered through 0.22 µm filter (Sigma) and 20 µl filtrates were injected into the HPLC system.

**Chromatographic and detection conditions**

A delivery pump Model HP 1050 (Hewlett-Packard) was used with a manual injector Model 7125 (Rheodyne, Berkeley). The analytical column ODS 2 (250 x 4.6 mm), particle size 5 µm (Hewlett-Packard) protected by guard column ODS 2 (20 x 2.1 mm), particle size 5 µm (Hewlett-Packard), was operated at a flow rate 1.4 ml/min and at a column temperature of 30°C.

The mobile phase was comprised of 0.15 M sodium dihydrogen phosphate, 0.1 mM EDTA, 0.5 mM sodium octanesulphonic acid, 10 % methanol (v/v) and 5 mM lithium chloride. The mobile phase was adjusted to pH 3.4 with phosphoric acid, fil-
tered through 0.22 μm filter (Sigma) and degassed with helium.

The electrochemical detector was Model HP 1049A (Hewlett-Packard) with a glassy carbon working electrode. A voltage setting of +0.65 V versus an Ag/AgCl reference electrode was used. The chromatographic peaks were integrated using a chromatointegrator (Esoft, Łódź). The quantification of the concentration of monoamines and their related metabolites in each sample was calculated from the integrated chromatographic peak area and expressed as ng/g wet tissue.

The concentrations of NA, DA, 5-HT and their respective metabolites MHPG, DOPAC, HVA and 5-HIAA were measured.

Methanol was purchased from Serva (Heidelberg). The other chemicals were purchased from Sigma Chemical Co (St. Louis, MO).

Statistics

The biochemical results were evaluated by a one-way ANOVA followed by Duncan’s multiple comparison test.

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tr>
<td>Regional brain distribution of NA, MHPG and MHPG/NA ratio</td>
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</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>Monoamine and metabolites content (ng/g; mean ±SEM)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA</td>
<td>MHPG</td>
<td>MHPG/NA</td>
</tr>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>801.03±111.98</td>
<td>21.50±3.24</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>1109.95±234.41</td>
<td>17.13±5.36</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>2071.26±331.47ab</td>
<td>22.24±4.37</td>
</tr>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>480.00±66.13</td>
<td>19.23±4.27</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>485.05±69.30</td>
<td>15.03±3.63</td>
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<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>906.49±102.50ab</td>
<td>21.65±5.15</td>
</tr>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>487.23±26.41</td>
<td>30.57±5.60</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>284.02±57.74a</td>
<td>7.48±4.30a</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>387.89±37.64</td>
<td>8.08±1.83a</td>
</tr>
<tr>
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<td>n=6</td>
<td>521.04±81.25</td>
<td>21.91±4.49</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>649.38±130.04</td>
<td>14.02±2.44</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>506.32±51.20</td>
<td>16.94±1.95</td>
</tr>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>372.85±46.18</td>
<td>15.80±1.86</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>377.83±94.17</td>
<td>24.08±4.35</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>518.30±144.89</td>
<td>11.46±5.08b</td>
</tr>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>323.85±21.59</td>
<td>22.01±2.82</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>476.61±69.28</td>
<td>30.75±10.73</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>351.07±52.20</td>
<td>12.22±3.94</td>
</tr>
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</table>

a, P<0.05 compared to single cats in predatory test; b, P<0.05 compared to dominants.
RESULTS

NA, MHPG concentrations and MHPG/NA ratio

The NA concentrations, shown in Table I, were significantly changed in the anterior \( [F(2,13)=8.23; P<0.005] \) and posterior \( [F(2,13)=9.19; P<0.003] \) hypothalamus among the groups studied. Duncan’s test indicated a significantly increased concentration of NA in the anterior and posterior hypothalamus of submissive cats in comparison with dominant cats (\( P<0.05 \), for both structures) and single preying cats (\( P<0.05 \), for both structures). A one-way ANOVA revealed changes in the content of NA in GC \( [F(2,13)=6.32; P<0.01] \) among the groups studied. A post hoc test indicated that the dominant position in a predatory situation produced a decrease in NA concentration in GC compared to single preying animals (\( P<0.05 \)). No significant differences in the distribution of NA in AM \( [F(2,13)=0.70; \text{NS}] \), HI \( [F(2,13)=0.69; \text{NS}] \) and CTX \( [F(2,13)=2.76; \text{NS}] \) were noted among the groups studied. MHPG concentrations, shown in Table I, were changed in GC \( [F(2,13)=9.20; P<0.005] \) and HI \( [F(2,13)=4.58; P<0.05] \). Duncan’s test revealed a decreased concentration of midbrain MHPG of dominant and submissive cats compared to single preying cats (\( P<0.05 \), for both groups); the content of

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain region</th>
<th>DA (ng/g; mean ±SEM)</th>
<th>HVA (ng/g; mean ±SEM)</th>
<th>DOPAC/DA</th>
<th>HVA/DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Single cats</td>
<td>n=6</td>
<td>1120.15±228.29</td>
<td>73.92±14.44</td>
<td>624.49±99.07</td>
<td>0.062±0.009</td>
</tr>
<tr>
<td>2. Dominants</td>
<td>n=5</td>
<td>2576.10±423.50</td>
<td>228.48±65.04</td>
<td>1098.90±174.49</td>
<td>0.084±0.014</td>
</tr>
<tr>
<td>3. Submissives</td>
<td>n=5</td>
<td>2790.94±576.92</td>
<td>193.90±38.53</td>
<td>1088.44±156.86</td>
<td>0.072±0.009</td>
</tr>
</tbody>
</table>

\(^a\text{, } P<0.05\) compared to single cats in predatory test; \(^b\text{, } P<0.05\) compared to dominants.
MHPG in HI of dominant cats was increased in comparison with the submissive animals \((P<0.05)\). The ANOVA showed a significantly changed MHPG/NA ratio in HI \([F(2,13)=4.41; P<0.03]\). The MHPG/NA ratio was higher in dominant cats in comparison with the other groups \((P<0.05)\) (Table I).

**DA, DOPAC and HVA concentrations, DOPAC/DA ratio and HVA/DA ratio**

The ANOVA showed significant differences in DA concentration in HI [\(F(2,13)=4.48; P<0.05\)] and posterior [\(F(2,13)=4.48; P<0.05\)] hypothalamus and hippocampus [\(F(2,13)=8.30; P<0.005\)] among the groups studied. Duncan’s test revealed a significant increase of DA content in HA and HP of dominant \((P<0.05, for both structures)\) and submissive \((P<0.05, for both structures)\) animals compared to DA content in respective structures of single preying cats (Table II); the dominant position in predatory hierarchy produced an elevation of hippocampal DA content in comparison with the other groups \((P<0.05, for both groups)\). The ANOVA revealed significant changes of DOPAC and HVA concentrations in HA \([F(2,13)=3.97; P<0.05 and F(2,13)=3.85; P<0.05, respectively]\). Duncan’s test indicated a significantly increased \((P<0.05)\) DOPAC and HVA content in HA of dominant and submissive animals compared to single preying cats.

\[
\begin{array}{cccc}
\text{TABLE III} & \\
\text{Regional brain distribution of 5-HT, 5-HIAA and 5-HIAA/HT ratio} & \\
\hline
\text{Group} & \text{Brain region} & \text{Monoamine and metabolites content (ng/g; mean ±SEM)} & \text{Ratio} \\
\hline
\text{1. Single cats} & n=6 & 2259.72±163.55 & 207.32±23.67 & 0.093±0.011 \\
\text{2. Dominants} & n=5 & 3735.22±674.78^a & 274.28±49.91 & 0.075±0.006 \\
\text{3. Submissives} & n=5 & 4526.34±563.50^a & 352.20±70.98 & 0.077±0.009 \\
\text{1. Single cats} & n=6 & 1953.14±229.38 & 258.51±29.51 & 0.135±0.013 \\
\text{2. Dominants} & n=5 & 1916.45±281.22 & 270.92±48.81 & 0.137±0.010 \\
\text{3. Submissives} & n=5 & 2351.30±221.36 & 329.35±68.52 & 0.134±0.018 \\
\text{1. Single cats} & n=6 & 2131.43±261.37 & 298.79±65.66 & 0.136±0.021 \\
\text{2. Dominants} & n=5 & 1804.72±342.20 & 297.25±57.51 & 0.168±0.032 \\
\text{3. Submissives} & n=5 & 1946.57±252.31 & 275.69±59.10 & 0.141±0.021 \\
\text{1. Single cats} & n=6 & 2787.73±271.22 & 252.51±28.36 & 0.092±0.009 \\
\text{2. Dominants} & n=5 & 3171.94±422.51 & 233.91±45.82 & 0.073±0.013 \\
\text{3. Submissives} & n=5 & 2635.81±328.89 & 278.80±52.42 & 0.103±0.007 \\
\text{1. Single cats} & n=6 & 2533.16±295.00 & 258.89±41.22 & 0.102±0.011 \\
\text{2. Dominants} & n=5 & 3503.84±354.36^a & 288.48±36.51 & 0.082±0.005 \\
\text{3. Submissives} & n=5 & 2359.83±125.54^b & 197.57±26.11 & 0.084±0.107 \\
\text{1. Single cats} & n=6 & 724.16±39.30 & 72.59±17.78 & 0.102±0.027 \\
\text{2. Dominants} & n=5 & 1138.05±234.86^a & 128.64±66.29 & 0.092±0.026 \\
\text{3. Submissives} & n=5 & 617.11±79.87^b & 60.90±15.55 & 0.098±0.021 \\
\end{array}
\]

\(^a\, P<0.05\, \text{compared to single cats in predatory test}; \, ^b\, P<0.05\, \text{compared to dominants.}
The one-way ANOVA revealed changes of HVA/DA ratio in HP \( F(2,13)=4.08; P<0.05 \). Post hoc comparisons showed a significantly increased HVA/DA ratio \( P<0.05 \) in HP of dominant and submissive animals compared to single preying cats (Table II).

### 5-HT, 5-HIAA concentrations and 5-HIAA/5-HT ratio

Significant changes in 5-HT concentrations were found in HA \( F(2,13)=5.87; P<0.02 \), HI \( F(2,13)=4.62; P<0.05 \) and CTX \( F(2,13)=3.90; P<0.05 \) among the groups studied (Table III). The content of 5-HT was elevated (Duncan’s test; \( P<0.05 \)) in HA of submissive and dominant cats compared to single preying animals. The dominant position of cats produced an elevation of hippocampal and cortical 5-HT content (Duncan’s test; \( P<0.05 \)) compared to animals from the other groups. 5-HIAA contents and 5- HIAA/5-HT ratio did not significantly differ in all the examined groups (Table III).

### DISCUSSION

The obtained data revealed a considerable differentiation and a complexity of the studied effects of predatory and social behaviour on regional brain distribution of monoamines.

The results of our investigation indicated that dominant and submissive cats differed from each other in the hypothalamic NA concentration and in hippocampal DA, 5- HT and MHPG content. The submissive position in the cat hierarchy scheme produced elevation of hypothalamic NA. This neurochemical result may indicate anxiety of submissive animals revealed by hierarchical relationships between the paired cats. The hypothalamus is regarded to be one of the basic structures involved in motivation and emotional behaviour (Blanchard and Blanchard 1988). Numerous data from other investigations speak for the fact that it is a psychological stimulus which enhances NA release (DeFeudis 1979). In another study, Eleftheriou and Church (1968) observed an initial rise of norepinephrine in the amygdala and hypothalamus in a mouse exposed to trained fighters. It has been postulated that the increased brain noradrenergic neuronal activity is closely related to the provocation of negative emotions, i.e., fear or anxiety (Stephens et al. 1986, Charney et al. 1990, Strzelczuk and Romaniuk 1995). Another study revealed that the animals exposed to a phobic stimulus and showing a marked avoidance had a significantly higher cortical noradrenaline content (Andrews et al. 1993).

In the present study, we demonstrated that the dominant position in the cat hierarchy scheme produced an elevation of hippocampal DA, 5-HT and MHPG concentration compared to submissive animals. Additionally, the presentation of another cat (submissive) decreased NA and MHPG concentrations in GC of dominant cats in predatory competition tests in comparison with the cats in predatory tests in the absence of another cat. These findings are likely to indicate increasing of emotional arousal in dominant cats in predatory competition. The elevation of hippocampal concentration of DA and 5-HT in dominant cats as observed in our study may be due to an intensified readiness to attack. The results of other laboratories show an involvement of hippocampus in facilitation of predatory attack (Siegel and Flynn 1968, Adamec 1991). On the other hand, the GC is one of the structures involved in the behavioural and cardiovascular components of defensive behaviour (Blanchard and Blanchard 1988).

The present data demonstrated that the predatory competition produced a considerable elevation of DA concentration in the hypothalamus of paired cats both dominant and submissive. Additionally, the hypothalamic concentrations of DA metabolites were also observed to be higher in dominant and submissive cats. This may indicate an increased metabolism of hypothalamic DA produced by the social situation in cats. It is worth noticing that the changes observed in monoamine concentration were located in the hypothalamus and occurred in consolidated and stable hierarchy in the predatory competition test. The process of consolidation of hierarchy is favored in conditions of a frequent con-
conflict (Rowell 1974) which may be due to the fact that cats are territorial animals, leading an asocial mode of life and meeting each other only to mate (Leyhausen 1979). The social interactions of paired cats at the beginning of predatory competition tests are accompanied by aggressive acts, namely: (1) hissing and growling, (2) paw striking, and (3) attack (Fonberg et al. 1985), while the hierarchy formation led to inhibition of aggressive acts (Rowell 1974). This fact may be the basis for an assumption that a steady hierarchy reduces conflicts (Rowell 1974) but does not reduce neurochemical mechanisms of aggression, i.e., it does not reduce DA concentration. The elevated concentration of DA was observed in the brain of fighting animals (Welch and Welch 1969, Kantak et al. 1984), especially in the hypothalamus (Kantak et al. 1984). The present findings are in agreement with the above mentioned observations.

In summary, the obtained data confirmed the considerable involvement of NA and DA systems in the regulation and modulation of predatory and social behaviour in cats. The submissive position elevates hypothalamic NA content in cats. This fact may due to anxiety which is induced by the presence of the dominant cat, the winner of the competitive predatory test.

It is worth noticing that a social interaction between two cats produced an increased DA activity in the hypothalamus. The hypothalamic DA system seems to be engaged especially in conspecific social behaviour because a social contact leads to a higher content of this catecholamine and its metabolites. This may suggest that predatory behaviour in the presence of another cat may be affected by both NA and DA mechanisms which interact with each other at the forebrain level. The dopamine-noradrenaline interactions have been documented in numerous instances (Antelman and Caggiula 1977). The engagement of the 5-HT system in the phenomena studied was not as marked as for catecholamines.

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