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Acute and Conditioned Blood Pressure Changes in Relation to Social and Psychosocial Stimuli in Rats

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FOKKEMA, D. S. AND J. M. KOOLHAAS. Acute and conditioned blood pressure changes in relation to social and psychosocial stimuli in rats. PHYSIOL BEHAV 34(1) 33-38, 1985.—The naturally occurring tendency to compete with other rats for territorial space has been used to study individual behavior characteristics and blood pressure reactivity to social stimuli in adult male TMD-S3 rats. The competitive characteristics of the individual rats are consistent in two different social situations (victory and defeat). Blood pressure responses during the victory of home territory rats over intruders was more pronounced in the more competitive animals. In addition to defeat by a trained fighter rat, the experiments were also psychosocially stimulated by the fighter while it was confined in a small wire mesh cage. The blood pressure response to this event was enhanced by the prior defeat of the test animal by the one now confined to the small cage. This response was more pronounced in competitive rats. This approach has potential as an animal model of etiological processes in socially induced hypertension.

Blood pressure Aggressive behavior Psychosocial stress Hypertension

A relation between the occurrence of cardiovascular diseases and personality was first noted by Friedman and Rosenman [3]. This observation was followed by extensive studies on coronary prone behavior [4]. Impressive knowledge has also been obtained from psychophysiological investigations on the regulation of blood pressure [13]. Despite these efforts the causal relationship between personality and the development of hypertension is largely unexplained, though evidence has been found that psychosocial factors are of importance [5,6]. Animal experiments may be very useful to investigate the relationship between psychosocial factors and cardiovascular physiology.

Unfortunately very few animals models deal with psychosocial factors. One of them is the model of Henry et al. [7]. They studied mice in a colony designed to induce frequent social interactions. Hypertension was found to occur in those animals that have a dominant position in the social hierarchy [2,7]. This relationship between dominance and blood pressure may be determined by the environmental situation purely or in combination with some individual feature in behavior or physiology. In order to analyse this we studied individual behavioral characteristics and blood pressure in a variety of social situations.

Both the level of social aggressive behavior and various physiological parameters such as higher plasma testosterone and lower corticosterone do differentiate victorious from defeated individual male rats [9,15]. Moreover social behavior of rats can easily be manipulated and studied quantitatively in the laboratory [9]. Therefore rats seem to be highly suitable to study blood pressure in relation to behavioral characteristics.

Three social situations were selected for this study: victory, defeat and psychosocial stimulation. When a male rat is kept in a sufficiently large cage, it will establish a territory, i.e., it will win fights against unfamiliar male intruder rats. Hence when using such territorial males as experimental rats their victory experience can be controlled. By introducing an experimental rat that has won on his home ground as an intruder into the territory of a trained fighter rat a defeat follows. In order to separate the physical stress from the psychosocial effects of such a defeat, we studied the blood pressure response of the experimental rat to presentation of the trained fighter rat when the latter was enclosed in a small wire mesh cage.

The present paper will demonstrate that animals with a certain behavioral characteristic which can be found consistently in the various social situations have higher blood pressure reactivity to that given social stimuli.

METHOD

Rats

Eleven male Tryon Maze Dull-S3 rats (originating from Cpb TNO, Zeist, The Netherlands and bred in the laboratory) were used as experimental animals. They were housed in the breeding colony 6-8 rats to a cage. Experimental rats were at least six months old. The average weight of the rats was 320±19 g at the start of the experiments.
Male WEzob rats (originating also from Cpb, TNO and bred in the laboratory; weight 250–350 g) were used as intruders in the experimental rat’s cage in order to elicit territorial behavior in the experimental TMD-S3 rats. WEzob rats are always subordinate to TMD-S3 rats [10]. To serve as dominants over experimental TMD-S3 rats some male TMD-S3 rats were selected for aggression and trained to fight. The weight of these trained fighters was 350–500 g.

**Housing**

The experimental rats and the trained fighters were housed permanently each in its own observation cage (80×55×50 cm) with one glass wall and a layer of wood shavings. Food and water were available ad lib. The light period was fixed at 12–12 hour reversed cycle. Experiments were always carried out during the dark period. In order to facilitate territoriality a female sterilized by ligation of the oviducts lived with the experimental rats. The female was removed for the periods of the actual experiment.

**Behavior**

Behavior was recorded on a keyboard processor which automatically calculates durations of 24 behavior elements. The behavior of the experimental animals was recorded during confrontation with opponents. The following behavioral categories were used (in parenthesis the relative duration in victory tests): (1) Rearing and scanning (40%); (2) Sniffing object (4%); (3) Investigating opponent (7.9%); (4) Genital sniffing (1.1%); (5) Grooming and scratching 22%; (6) Social and aggressive grooming (0.6%); (7) Fixating and nosing (0.6%); (8) Full aggressive posture (0.9%); (9) Moving towards opponent (3.9%); (10) Chasing (1.2%); (11) Moving away from opponent (2.2%); (12) Flight (0.6%); (13) Lateral threat (3.8%); (14) Keep off (0.8%); (15) Upright posture (0.6%); (16) Mutual upright (3.6%); (17) (Bite and Kick) attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (0.6%); (20) Freezing (0.6%); (21) Resting (0.9%); (22) Crawling under or over the opponent (0.1%); (23) Mounting (0.3%) and (24) Other behavior elements (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%) These behaviors are defined in such a way [10,11] that the animal always performs one and never more than one behavior at a time. The durations are expressed as percentages of the total duration of the confrontation (relative time). The term aggressive behavior is used for the elements threat, bite and kick, attack (1.2%); (18) Keep down (1.7%); (19) Submissive posture (2.4%)

**Blood Pressure Recording**

The experimental rats were canulated with silicon tube (0.6 mm Dow Corning silastic 602–105) directly into the abdominal aorta, just anterior of the ileac arteries. Cannula tips were 6 mm long and pointed upstream. They were fastened by means of a loose loop of thread (4–0) around the aorta. Implantation of the catheter was done through a midline incision in the abdomen while the rat was under complete ether anesthesia. The end of the cannula was passed subcutaneously to the head where it was connected to a thick piece of silicon tube (i.d. 0.5 mm, o.d. 3 mm, 1.5 cm long) by means of a curved piece of injection needle which was fixed onto the skull with screws and dental cement. The thick piece of silicon tube served for easy connection to recording devices. It could be clipped during disconnecting and closing of the cannula to prevent outflow of blood. Between the experiments it was protected by an aluminum screw cap.

To make a continuous blood pressure recording the animal’s headpiece was connected to a pressure transducer (Honeywell 130 PC) by means of polyethylene tube (i.d. 1.5 mm) in which two swivel joints were incorporated. A spring kept the cannula tight during vertical movements. This connection did not restrict the rat’s movements inside the cage. The signal from the pressure transducer was visible on an oscilloscope and fed into a Wekagraph 250 recorder. The latter was used to make written registrations of 25 seconds duration at the times mentioned in the procedure section. Calculations were performed by digitizing (Hewlett Packard 9874A) data from the registrations and feeding them into a desktop computer (Hewlett Packard 9835A).

In order to calculate a value of mean blood pressure, 25 samples of the systolic and of the corresponding diastolic blood pressure values were taken once every second. From these samples the mean was calculated. Baseline blood pressure was determined daily in the resting rat immediately prior to the behavioral testing. This recording was made 15 minutes or more after handling—i.e., connecting to the transducer. A blood pressure response was defined as the difference between the mean blood pressure during a certain stimulus (pause value in victory and defeat tests) and the previously recorded baseline mean blood pressure in the resting rat.

**Procedure**

The experimental rats were given a week to become territorial in their home observation cages. Subsequently, they were cannulated and given another week to recover. During this week they were connected to the pressure transducer several times to habituate to the procedure. Three types of tests were used: victory, defeat and psychosocial stimulation. Every experimental rat was run through the complete series of tests (Table 1). Five daily victory tests with intruders were followed by a single defeat by the trained fighter on day six. Thereafter on three subsequent days (days 7, 8 and 9) again victory tests with intruders were performed. Psychosocial stimulation by exposure to the caged trained fighter was carried out on the three days before and on the three days after the defeat, each time prior to a victory test.

Victory tests were performed in the home cage. An intruder rat was put in the cage of the experimental rat for 10 minutes during which time they could interact freely (Table 1). If there was a fight the experimental rat always won because, as already mentioned, S3 rats are always dominant over WEzob males [10]. Intruders never start fights. During the test the behavior of the experimental rat was recorded continuously. Twenty-five second blood pressure recordings were taken immediately prior to the test (the baseline), in the first interval in which the rat sat motionless after the first attack. In case there was no attack these were made after intensive contact with the opponent (pause value), and at 1, 5, and 10 and 30 minutes after the intruder had been removed.

Defeat tests were done in the home cage of the trained fighters. After the experimental rat had been put in the cage of the fighter which had been removed temporarily, he was given 15 min time to recover from handling (Table 1). Then the trained fighter was introduced for 30 min. After the confrontation the experimental rat was placed in his home cage. Behavior of the experimental rat was recorded throughout the 30 min of confrontation and blood pressure was measured in the home cage prior to the test, after 15 min in the fighter’s cage, in the first interval in which the rat sat
### TABLE 1

**EXPERIMENTAL PROCEDURE**

<table>
<thead>
<tr>
<th>Test</th>
<th>Victory 1-5, 7-9</th>
<th>Defeat 6</th>
<th>Psychosocial 3-5, 7-9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfomed on day No.</td>
<td>time</td>
<td>time</td>
<td>time</td>
</tr>
<tr>
<td>Event</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat in home cage connected to recording device</td>
<td>-16</td>
<td>-30</td>
<td></td>
</tr>
<tr>
<td>Baseline BP recording</td>
<td>-1</td>
<td>-17</td>
<td></td>
</tr>
<tr>
<td>Transfer of exp. rat to fighter's cage</td>
<td>-16</td>
<td>-16</td>
<td></td>
</tr>
<tr>
<td>BP recording in fighter's cage</td>
<td>-1</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>Opponent rat is introduced, start of behav. rec.</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Encaged fighter rat is introduced, BP recording</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP recording previous to fight</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BP recording after attack</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Opponent is removed (end of behav. rec.)</td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Exp. rat is transferred to home cage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP recordings</td>
<td>11, 15</td>
<td>31, 35</td>
<td></td>
</tr>
<tr>
<td>BP recordings</td>
<td>20, 40</td>
<td>40, 60</td>
<td></td>
</tr>
</tbody>
</table>

Time in minutes, BP=blood pressure, x=pause value (see text).

---

**FIG. 1.** Individual changes in baseline mean blood pressure (mm Hg). The average of six daily measurements before defeat is compared with the first day after defeat.

**FIG. 2.** Mean blood pressure (mm Hg) during a ten minute victory test (solid black bar). Mean and standard deviation of five victories in each of eleven rats.

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motionless after the first attack (pause value) and at 1, 5, 10 and 30 min after the experimental rat has been replaced in his home cage. Judging from the performance of submissive or freezing behavior experimental rats were properly defeated.

For the psychosocial test situation, the experimental rat was placed in the cage of the trained fighter which had been removed temporarily. After the experimental rat has been in the home cage of the fighter for 15 min a wire cage (12×12×22 cm) containing the fighter was presented for 1 min (Table 1). Blood pressure was recorded in the experimental animal's home cage, after 15 min in the fighter's cage and during the presentation of the trained fighter. The encaged fighter in the psychosocial test situation was always the same animal that had been used to defeat the experimental rat. The psychosocial tests were only done in nine out of the original eleven experimental rats.

**Statistics**

Behavioral data are expressed as relative time and standard deviation, mean blood pressure as mm Hg and standard deviation. To examine whether an association exists between the two, Spearman's rank correlation coefficients were calculated [16]. In case tests were replicated the average of the data was calculated and used unless otherwise mentioned. Blood pressure values were approximately normally distributed. Differences between days (repeated measurements) were analysed with a t-test for paired com-
comparisons testing values for a specific day against the average of other days or with a two-way ANOVA without replications. The effect of defeat experience was tested with a t-test for paired comparisons comparing a single day data with the average of pre-treatment values. A two-way ANOVA using pre-treatment and post-treatment as blocks with days as replications within the blocks, was applied to test an effect over more days. Interaction is only mentioned when significant.

RESULTS

Blood Pressure

The baseline mean arterial blood pressure of the experimental rats was 91±4.9 mm Hg. Despite variations in baseline up to 30 mm Hg within individuals among repeated measurements there was a significant difference in baseline between rats (two-way ANOVA without replication, F(10,30)=3.16, p<0.005).

No significant change in baseline blood pressure was found following repeated victory (two-way ANOVA; F(5,50)=0.83). However, the day after the defeat the blood pressure was 86±7.3 mm Hg which is below the average of six previous recordings (paired t-test, two tailed p<0.10) (Fig. 1). The two subsequent daily recordings of baseline blood pressure after defeat did not differ from pre-defeat values (two-way ANOVA; F(1,79)=1.62).

During social confrontations the blood pressure of the experimental rat became elevated. The average blood pressure response in five victory tests before defeat was 13±6.6 mm Hg, which results in a mean blood pressure of 103±11 mm Hg (Fig. 2). After defeat the victory response was 12±6.3 mm Hg which was not significantly different (two-way ANOVA; F(1,69)=2.33) from the one before defeat.

Five minutes after the intruder had been removed the blood pressure was still elevated in eight out of ten rats. The average blood pressure (n=10 rats) was 94±10.0 mm Hg which is significantly higher than baseline when tested over the error (two-way ANOVA; F(1,60)=5.97, p<0.05) but not when tested over the interaction (F(1,60)=2.73, n.s., interaction F(9,60)=2.19, p<0.05). Ten minutes after the confrontation the blood pressure did not differ significantly from baseline (two-way ANOVA; F(1,54)=2.38).

The average blood pressure response of an experimental animal to defeat by a dominant rat was an increase of 25±8.1 mm Hg.

The average blood pressure increase to three psychosocial stimulations before defeat was 13±4.8 mm Hg and after defeat 20±12.2 mm Hg. All rats had a higher blood pressure response one day after defeat compared to the pre-defeat responses to psychosocial stimulation. When the data are treated as two blocks (before and after defeat) with three replications each, there is a significant effect of defeat (two-way ANOVA; F(1,34)=5.34, p<0.05). The average of pre-defeat responses was also compared with each post-defeat day separately by means of a paired t-test; only on the first day after defeat is the response (25±11 mm Hg) significantly elevated, p<0.01 (Fig. 3).

The blood pressure response to the psychosocial stimulus consists partly of a response to the fighters cage. However, the response to the presentation of the fighter was always higher than merely to the exposure to the fighters cage (t-test, p<0.01). The day after defeat the response to the fighters cage was higher than the average of the three pre-defeat responses (t-test, p<0.05).

In addition to the response, the absolute mean blood pressure during the psychosocial stimulation was higher on the day after defeat (111±13.7 mm Hg) than the average of the three pre-defeat values (102±5.1 mm Hg) (paired t-test, p<0.05). The absolute mean blood pressure of the experimental rat in the mere territory of the trained fighter, before the latter was reintroduced, was not significantly different before (97±5.6 mm Hg) and after (99±7.3 mm Hg) defeat (paired t-test).

Behavior

During victory tests the experimental rat spent 6.2±6 percent of the confrontation time in aggressive behavior towards the intruder rat. Hardly any defensive behavior was observed (0.6±1% Keep off). For convenience the average durations of all recorded behaviors during pre-defeat victory tests are incorporated in the behaviors list in the Method section. Behavioral performance stabilized after the first two tests. Therefore, individual differences in the performance of aggressive behavior were tested for days 3, 4 and 5. The difference between individuals was significant (two-way ANOVA without replications; F(10,22)=4.14, p<0.005).

During defeat by a fighter the experimental rat hardly performed any aggressive behavior (1±2.1%). Defensive behavior towards the opponent was 26.5±2.3 percent of the total duration of the confrontation.

The relative measure of the defensive behavior during defeat was highly correlated with the measure of the aggressive behavior directed towards intruders during victory (R=0.86). The more aggressive rats in their home cage were the more defensive when confronted with a trained fighter while other experimental rats were more passive in both situations.

The experience of defeat was not reflected in a change of aggressive behavior during victory tests (two-way ANOVA; F(1,54)=0.02).

The Relationship Between Behavior and Blood Pressure

The duration of the single elements of social behavior as measured in victory and defeat failed to correlate signifi-
cantly with the baseline blood pressure. However, during victory the individual blood pressure response averaged over five tests correlated positively with the relative durations of three behavioral elements: threat (R=0.64), bite and kick attack (R=0.81) and chase (R=0.81).

For convenience these behavioral elements are covered by the term aggressive behavior. Together they account for 70% of the aggressive behavior of the rat. The correlation of this score of aggression with the blood pressure response is 0.76. The more aggressive behavior was performed by the experimental rat, the higher his blood pressure response was to the victory test. Apart from the three behavior elements just mentioned others had positive correlations just under the 5% significance level (R=0.63). These were: mutual upright (R=0.58), moving towards (R=0.52), full aggressive posture (R=0.51) and keep down (R=0.51).

The duration of defensive behavioral elements during defeat did not correlate significantly with the corresponding blood pressure response. However, the durations of four behavioral elements during defeat did correlate significantly with the blood pressure response during victory: upright (R=0.75), flight (R=0.88), submissive posture (R=0.72) and sniffing objects (R = -0.68). The sum of the three positively correlated elements is referred to as defensive behavior. The correlation of this defensive score during defeat with the blood pressure response during victory was 0.90. The rats with higher blood pressure responses during victory performed more defensive behavior in defeat situations. Hence, blood pressure responses during victory correlate significantly with behavior during both victory and defeat.

As already mentioned the blood pressure response of the experimental rats to psychosocial stimulation was elevated after defeat as compared to before (Fig. 3). The average of the defeat-induced elevations of the response on the three post-defeat days was significantly correlated with the aggressive behavior during victory tests (R=0.84). Not on all post-defeat days separately a significant correlation was found. For unknown reasons it appeared to be very low on the second day. No such correlation was found with responses to the fighters cage.

In general, the more aggressive the rat is, the more pronounced is the defeat-induced elevation of the blood pressure response to psychosocial stimulation.

DISCUSSION

During and following social interactions in rats immediate individual blood pressure responses and slight alterations in baseline blood pressure have been observed. The major finding of the present study is that of a non-selected population the individual differences between male rats in semi-natural territorial social behavior are highly correlated with the magnitude of the blood pressure response during this behavior. The more aggressive rats have the higher blood pressure responses. Psychosocial stimulation by the caged victorious fighter rat produces as strong transient blood pressure elevations as a social interaction involving physical contact. Three out of eleven rats have an even higher response than during the actual confrontation. Figure 4 summarizes the findings on the magnitude of the blood pressure response.

Since the procedure of the stimulus presentation and the stimulus rat (trained fighter) involved are the same before and after the defeat this can be considered as a conditioned response. As in the responses to victory this conditioned blood pressure response differentiates significantly between individual rats. This response is more pronounced in rats that are aggressive in victory tests.

Thus, in two different social situations the blood pressure reactivity appears to be related to aggressive behavior during victory. This aggressive behavior is also correlated with defensive behavior during defeat. These two combinations suggest that high blood pressure responses occur in rats that are competitive when challenged by an intruder and also when threatened by a dominant. Apparently the intensity of the behavior that is to be performed and the accompanying blood pressure responses are dependent on the individual character of an animal rather than on the given psychosocial environment. The competitive rats always seem to control their environment, either by aggressive behavior in home cage fights or by defensive behavior against a dominant in contrast to the generally passive behavior of other rats.

No individual differentiation related to behavior is found in blood pressure reactivity during defeat. Apparently, defeat stimulates blood pressure irrespective of behavioral characteristics whereas the presence of an intruder rat in the home territory does activate the cardiovascular system differentially in relation to the behavior of the animal. An important difference between these two situations may be the possibility for a successful behavioral strategy in the victory situation, whereas during defeat no escape is possible and most behavior may be performed in reaction to actions of the trained fighter rat. In other words: maybe individual differences in appreciation of a stimulus situation occur only when successful active behavior is possible.

This idea is supported by studies in humans in which cardiovascular responses in challenging but solvable situations were compared with those during an impossible task. Behavioral performance was similar in the two situations, but individual differences in cardiovascular responsiveness were reduced during the impossible task [12]. So test situations without a possibility for active behavioral strategy seem to be less useful for the study of individual cardiovascular reactivity.

Enhanced motor activity and consequent higher metabolic need may be a factor of significance to the blood pressure response [14]. Also in victory tests in the present study individual differences in motor activity may account for individual blood pressure reactivities. However, the
difference in reaction to the psychosocial stimulus before and after defeat suggests that psychosocial factors may be of importance. Hardly any activity occurs during this stimulation. Therefore it seems unlikely that metabolic requirements play a role to such an extent that they can fully account for the blood pressure response.

With respect to baseline blood pressure, the present study surprisingly shows that with the loss of a fight the blood pressure decreases. Recent experiments replicate this result. The decrease in baseline cannot explain the higher response to a psychosocial stimulus on the first day after defeat compared to pre-defeat, since also the absolute mean blood pressure is elevated at that time. Victory does not have a demonstrable effect on baseline blood pressure, neither does psychosocial stimulation. To what extent these different social situations, when repeated frequently, affect baseline blood pressure on the long term is a matter for further research. However, observations of persistent hypertension in the dominant members of mouse and rat colonies are consistent with the present data showing a higher blood pressure reactivity in competitive animals [1, 2, 7]. A similar observation is the greater hypertension of better performing squirrel monkeys during operant conditioning [8].

The results on the individual differentiation of behavior and blood pressure of the group of rats in this study show a similarity with various observations in man. The risk for cardiovascular disease in man is correlated with type A coronary prone behavior which among other characteristics involves competitive achievement striving [4]. Also the responsiveness of behavioral and cardiovascular parameters is believed to be of importance to the development of human hypertension [6]. The present technique for the study of the rats social behavior provides a good animal model of etiological processes in human hypertension because it differentiates between individuals with respect to behavior in concordance with individual blood pressure responsiveness.

REFERENCES