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Multimetric statistical analysis of behavior in mice selected for high and low levels of isolation-induced male aggression

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Abstract

Behavioral observations as a matrix of probabilistic changes of postures and acts are multiple measurements that could introduce variability to statistical analysis. We propose the multimetric statistical algorithm that supplements the linear analysis of variance by pair correlation, factor and discriminant function analyses. Although these methods were utilized mostly in behavioral studies, the combined use in frame of one behavioral test was not done before. In present study statistical techniques were applied to analyze social behavior in Turku aggressive (TA) and Turku non-aggressive (TNA) mouse lines, bidirectional selected for offensive aggression towards an unknown male.

Each statistical technique amplified new details of mouse behavioral profiles that give possibility to describe TA and TNA subjects in terms of Cloninger's model of personality. Also, it was identified that TA mice displayed fighting–biting aggression while TNA mice demonstrated immobile defensive strategy. Hypothetical discriminant formula was found for each mouse behavioral genotype that might be used to identify behavioral profile and line affiliation of unknown subjects.

Keywords: Aggression; Ethological method; Mice; Statistical analysis

1. Introduction

Experimental psychopharmacology is based on ethological observation, description and measurement of behavioral elements in modeling of mental illnesses (Dixon, 1986; Dixon et al., 1989; Dixon and Fisch, 1998; Ferrari et al., 1998; Podhorna and Krsiak, 2000; Miczek et al., 2001). The practical mission is to screen new drugs or mutantions in mice and to collect information concerning novel theories of molecular mechanisms implicated (Lipska and Weinberger, 2000; Crawley, 2004).

Any behavioral observation formally presented as a pool of sequences and durations of discrete behavioral elements (Poshivalov and Khodko, 1984; Griebel et al., 1999; Troisi and Moles, 1999; Leighty et al., 2004) that requires special analysis to evaluate all of the parameters as an integrated framework. This set of parameters is a matrix of probabilistic changes of behavioral measurements that requires a statistical analyses of higher level than mere descriptive statistics and analysis of variance techniques (Leighty et al., 2004). To formally assess the quality of studies and to evaluate the collected data sets we conducted integration of the multimetric analysis, including step by step pair correlation, factor (principal components analysis) and discriminant function analyses into the data processing. This study has a special structure that allows to determine whether the statistical tools used make improvements in data analysis or just produce overlapping results. In addition, to optimize the method of data evaluation, discriminant function based on dependent variables (parameters of behavioral elements) was compared to discriminant function analysis based on emerged factors.

Similar multimetric statistical analysis utilizing all three techniques was conducted to analyze the set of independent parameters within the series of tests (Leighty et al., 2004). In this study we suggest to analyze all the parameters in frame of one behavioral test. Until recently, sequence (Brain et al., 1985; Jones and Brain, 1985) or discrete model (Poshivalov and Khodko, 1984; Poshivalov et al., 1988) analyses that are

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in fact Hierarchical Cluster analysis, were the most prevalent statistical technique to analyze immersed social structure in experimental psychopharmacology. However, some limitations of this analysis create the opportunity to explore the use of different techniques for data management.

Thus, pair correlation analysis although reflects the association between any two elements, does not clarify cause-and-effect relations in full and therefore is low informative when used single-handedly. Factor analysis which also describes the relationship between different variables, draws space vectors of each behavioral element towards main statistically determined direction producing cumulative entities called "factors" (Griebel et al., 1996; Boguszewski and Zagrodzka, 2002; Kanari et al., 2005; Porrini et al., 2005; Brenes Saenz et al., 2006). Discriminant functional analysis as a multivariate statistical technique, allows to distinguish between pre-defined subject groups as well as to determine unclassified subjects, and to pull out principal variables for group differentiation (Leighty et al., 2004). Moreover, discriminant function analysis while extracting the principal variables, also derives a mathematical description (formula) of specific behavioral profile. Despite of illustrative abilities of the technique it was mostly used to analyze data of psychiatric studies (Pokorny et al., 1999; Stip et al., 1999). Recent studies showed that it could be successfully utilized for evaluating the experimental results as well (Rogers et al., 2001; Salome et al., 2002).

Suggested multimetric statistical method was applied to social behavior of Turku aggressive (TA) mice with acute form of aggression towards an unknown partner and Turku non-aggressive (TNA) mice displaying feebly marked aggressive behavior. Aggression is a common behavioral pattern that animals share with humans and therefore analyzing details of aggression structure in integrated behavior of mice could provide explanation for similar human patterns (Dixon and Fisch, 1998). The development of these mouse lines started in 1959 (Lagerspetz, 1964) with the focus on effects of environmental conditions (Nyberg et al., 2004) or individual mouse characteristics such as locomotor activity, learning abilities, level of anxiety and its neurochemical and endocrinological correlates (Sandnabba, 1996; Nyberg et al., 2003). However, detailed analysis of line specific behavioral profile was omitted although the relations between agonistic, or competitive, pattern and other behavioral patterns might be informative. It is well established that TA mice demonstrate a high level of aggression toward the partner, appear more mobile and less anxious than TNA mice (Sandnabba, 1996; Nyberg et al., 2003) whereas the mechanisms underlying the morbidness of their aggression are still to be explained. Moreover, behavioral profile of TNA mice appears unclear since the absence of aggressive pattern in mice might be described as alternative copy strategy (Benus et al., 1991) or depressive-like condition. Variety of statistical techniques was used to analyze the behavioral profiles and to evaluate the adequacy of the data analysis system. Furthermore, multimetric statistical analysis was used to assess whether utilizing additional statistic techniques could discriminate between behavioral profiles more effectively compared to analysis of variance technique alone.

2. Method

The 19 TA and 18 TNA mice of the 75th generation by male selection from a Swiss albino foundation stock in Turku, Finland (Lagerspetz, 1964; Sandnabba, 1996; Nyberg et al., 2003) were housed individually in 22 cm × 17 cm × 14 cm clear polycarbonate cages with wire lids after weaning at 21 days of age. Isolated house condition for TA and TNA male is standard and all males of both lines are kept always in isolation after weaning. Swiss albino mice (SW; $n = 18$), that exhibit both aggressive or non-aggressive potencies (Sandnabba, 1996), were used for comparison as the heterogeneous background line for TA and TNA mice (Lagerspetz, 1964). Such behavioral structure representation would facilitate the interpretation of TA and TNA mouse specificity. Moreover, SW males were used as partners for TA and TNA mice which was required to establish SW behavioral profile.

Different mouse lines were kept in separate rooms. The behavior of 3 months aged males towards unknown SW partner were observed on neutral territory for 7 min (see details in Section 2.1). SW males used as a partner or as a subject were housed in three-member groups in 38 cm × 15 cm × 22 cm cages. Different condition of housing between SW and selected TA and TNA mice was not taken in consideration since it was hold as a standard for whole period of selection.

Animals housed in air-conditioned rooms (23 ± 1 °C and relative humidity of 50–60%) on 12 h daylight cycle with lights on at 07:00 h. Tap water and standard laboratory chow (Lab For) were available ad libitum. The experimental procedures were approved by the Ethical Committee for Laboratory Animal Research at the Åbo Akademi University.

2.1. Behavioral testing

Encounters of TA or TNA or SW male with unknown SW partners that took place in a neutral round glass arena (18.5 cm in diameter and 11 cm high) without bedding were video-recorded for 7 min after placing both subject and partner (both males) in. Duration of the encounter was chosen according to time of first procedure to evaluate the level of aggression in new TA and TNA mouse generations after weaning (Sandnabba, 1996). Under condition of new territory the interaction for both subject and its partner was considered in terms of subject–partner paradigm rather than resident–intruder.

If SW partner got any observable wounds from the opponent it was taken out of the experiment and allowed to heal. Since the behavior of partners was not taken into consideration, the injured partners were substituted for healthy mice. The arena was cleaned before each encounter.

The video records were observed subsequently using a computer-assisted data acquisition system (Ethograph, 2.06, Ritec, St. Petersburg, Russia) (Poshivalov et al., 1988; Vekovischeva et al., 2004) to register duration and frequency of majority observable elements. Some elements such as tail rattling, tremor and palpebral closure were fixed as secondary, in parallel with primary elements since these emotional expressions had to accompanied by any act or posture. The list of

Table 1
Definitions of behavioral categories and related elements

Behavior categories	Behavioral elements	Contraction
Aggression		
Consummate aggression	Fighting	F
	Biting	B
	Boxing with partner	Bx
Ambivalent aggression	Rushing to the attack	RshA
	Tail rattling #	TlRt
	Threat	Thr
	Circling around partner*	Cir
Defense	Kicking of the partner	Kk
	Avoidance*	Av
	Posture on the back	Bk
	Evasion	Ev
	Freezing	Fz
Partner exploration	Sniffing of partner's body	SnPr
	Sniffing of partner's anogenital area	SxSnPr
	Grooming of the partner	GrPr
Ambivalent stances	Lateral stance	LS
	Vertical stance	VS
Locomotion*	Quick locomotion	QL
	Locomotion with sniffing	LSn
	Chasing	Ch
	Approach	App
Non-aggressive contacts with a partner	Climbing over	ClO
	Climbing under	ClU
	Toss of partner	TPr
	Sexual contacts with partner	SxPr
	Passive contact with partner	Pass
	Grouping together with partner	Grp
Self-grooming		Gr
Rears		R
Sitting with sniffing		StSn
Other behavior	Rotation	rt
	Stretched attend posture	SAP
	Sitting	st
	Scratch	sc
	Tremor #	tr
	Palpebral closure #	plp
	Shake	sh
	Jump	j
	Lying with sniffing	ln
	Feeding	fd

Notes: (*) the elements were also included in locomotion category; (#) the secondary elements that were registered in parallel with any primary elements. Registration Pass or Grp was dependent on the initiator, partner or subject, of the physical contact.

observable elements is presented in Table 1. All behavioral elements were scored for total duration, medial duration (MDR), total frequency and relative frequency (RF) for each animal. Values of MDR and RF parameters were chosen for multimetric statistical analysis since they are considered independent from one another as well as from test duration.

2.2. Statistical analysis

Statistical analysis was conducted using SPSS v. 12.0 statistical package. Screening data and comparison of MDRs and RFs of each behavioral variable (behavioral elements or behavioral categories) between mouse groups were provided using multivariate analysis of variance (one-way ANOVA). Levene's test of homogeneity of variances was applied. Scheffe or Tukey tests, when variances were homogeneous, or Games–Howell test when non-homogeneous, were chosen for post hoc analysis of between-group comparisons (only when ANOVA revealed significant main effects). Null hypothesis was rejected at the $p < 0.05$ level.

2.2.1. Correlation analysis

Nonparametric bivariate correlations procedure (Spearman's rho) was used to analyze the significance of pair correlations between behavioral parameters. Statistical correlations reflect the extent of association between any two parameters; hence it involves the pair-wise comparison data. We determined correlations within behavioral structures of each mouse line, focusing on the highest level of the correlations (from 0.6 to 1.0) only.

2.2.2. Factor analysis

Factor analysis is a data reduction method which can be used to reveal underlying relationships among variables. It is a complex mathematical operation by which variables (MDR and RF in our case) were organized into the composite entities called "factors". The factor loading for each behavioral measure provides an estimate of how well that parameter reflects a particular variable. Thus, only factor parameters with loading values exceeding 0.50 were reported while loadings of less than 0.5 suggests that a particular parameter is a poor measure of a variable and were ignored.

MDRs and RFs parameters of behavioral elements were analyzed firstly for the entire set of mice (with Equamax rotation and Kaiser normalization (Child, 1971)) and, secondary, for each mouse line independently (without rotation). The principal components extraction method was used. We avoided use of rotation procedure to compare mouse lines because rotation prohibits the comparison of matrixes.The weight of each rotated factor was calculated for each subject and compared by multivariate analysis of variance (one-way ANOVA). Levene's test of homogeneity of variances was applied. Scheffe or Tukey tests, when variances were homogeneous, or Games–Howell test when non-homogeneous, were employed for post hoc analysis of between-group comparisons (only when ANOVA revealed significant main effects). Null hypothesis was rejected at the $p < 0.05$ level. Factor identified as different by analysis of variance were compared with factors found as discriminate by discriminant function analysis.The unrotated factor loading patterns reproduces most accurately the expected segregation of variables by factor that was compared qualitatively between lines (Leighty et al., 2004).

2.2.3. Discriminant function analysis

Discriminant function analysis is a multivariate statistical technique which is typically used: (1) to distinguish between predefined groups on the basis of differences in multiple measurements, (2) to identify the variables which contribute significantly to group differences and, thus best predict group membership, (3) to determine an optimal manner for distinguishing between groups, and (4) to determine group membership of the unclassified individuals (Leighty et al., 2004). Moreover, this technique allows to derive a mathematical formula of specific behavioral profile.

We provided two types of discriminant functions. One was based on the entire set of RFs and MDRs of behavioral elements; another was based on factors emerged by factor analysis for the whole set of mice. We used SPSS classification discriminant procedure based on Mahalanobis distance. Mahalanobis distance is a measure of how much a case's value on the independent variables differs from the average of all cases in *n*-dimensional space of *n*-variables. It is more correct to describe this point as a specific centroid of the classification group. We classify subjects in a group by its Mahalanobis distances since it is shorter to the centroid of that group than to those of any other alternative group. A large Mahalanobis distance identifies a case by showing extreme values on one or more of the independent variables. We used the "Stepwise-Forward" procedure that generates a mathematical model that incorporates specific variables chosen by iterative selection and testing. The stepwise-forward approach begins with no variables in the model, then constructs a model by including (or, sometimes, removing) variables one by one until all variables are examined, while conducting significance test at each step (entry $F = 3.84$, removal $F = 2.71$).

3. Results

3.1. Analysis of variance

Significant differences between behavioral categories are presented in Table 2. Almost for every variable the value for Levene's test statistics was less than 0.05 therefore Games–Howell post hoc test was additionally employed for data analysis. Homogeneous variables analyzed by Levene's test including such categories as parameters of consummate aggression (MDR), non-aggressive contacts with a partner (MDR) and self-grooming (MDR) were further subjected to Tukey post hoc test. Values of test statistics of consummate and ambivalent aggressions (MDR and RF) were higher in TA mice than in TNA and SW mice. Parameters of defense (MDR and RF), locomotion (RF) and rears (RF) were similar in TA and TNA mice. Other variables of TA and TNA mouse behavior exhibited several differences. For example, SW mice showed lower value for parameters of defense (MDR and RF), locomotion (MDR) and partner exploration (RF) than TA and TNA mice while exhibiting higher level of rears (RF). The level of risk-assessment behavior SAP (MDR and RF) was the highest in TNA mice and the lowest in TA mice; while SW mice showed the middle value for this factor (data not shown). Significant differences between all three lines such as TA > TNA > SW, were found for locomotion (MDR) and partner exploration (RF) behavioral categories.

Table 2
Differences in medial durations and relative frequencies of behavioral categories and related elements

Variables	Measured parameters	*F*	*P*	Observed Power (*a*)	Related elements	Differences (post hoc test)
Consummate aggression	MDR	135.02	.000	1.00	F, B, Bx	TA > (SW = TNA)
	RF	68.27	.000	1.00	F, B, Bx	TA > (SW = TNA)
Ambivalent aggression	MDR	197.05	.000	1.00	RshA, TlRt, Thr, Cir	TA > (SW = TNA)
	RF	118.93	.000	1.00	RshA, TlRt, Thr, Cir	TA > (SW = TNA)
Defense	MDR	12.41	.000	0.99	Av, Kk,Fz	(TA = TNA) > SW
	RF	17.82	.000	1.00	–	(TA = TNA) > SW
Partner exploration	MDR	7.33	.002	0.93	SnPr	(TA = SW)<(TNA = SW)
	RF	44.38	.000	1.00	SnPr	TNA > TA > SW
Non-aggressive contacts with the partner	MDR	6.74	.002	.90	ClU, Pass	TNA > (TA = SW)
	RF	4.17	.021	0.71	ClU, Pass	TNA > (TA = SW)
Ambivalent stances	MDR	1.36	.265	0.28		
	RF	103.87	.000	1.00	LS, VS	TA > (SW = TNA)
Locomotion	MDR	51.17	.000	1.00	Av	TA > TNA > SW
	RF	19.64	.000	1.00	LSn, Av, App	(TA = TNA) > (SW = TNA)
Rears	MDR	9.61	.000	0.98		(TA = SW) < TNA
	RF	25.36	.000	1.00		(TA = TNA) < SW
Self-grooming	MDR	3.48	.038	0.63		(TA = SW) < (TNA = SW)
	RF	2.22	.119	0.43		
Other behavior	MD	19.96	.000	1.00	SAP	TA < SW < TNA
	RF	33.09	.000	1.00	SAP	TA < SW < TNA
Sitting with sniffing	MDR	8.40	.001	0.96		(TA = SW) < TNA
	RF	12.17	.000	0.99		(TA = SW) < TNA

Note: The abbreviations of the behavioral elements are decoded in Table 1. MDR: medial duration; RF: relative frequency.

3.2. Correlation analysis

The behavioral structure of TA mice consists of correlations between aggressive elements as well as between aggressive elements and other behavioral patterns excluding defense. The elements of ambivalent stances showed high correlation with consummate aggression such as “fighting”, “biting” and “boxing”. Within consummate aggressive pattern “fighting” correlated with “biting”.

Within the structure of TNA mice, defensive and aggressive elements showed high correlation with many elements of other behavioral patterns but not between each other. Ambivalent stances, in turn, correlated with consummate aggression “boxing” and defensive element “kicking”, described as a push by hind legs. Ambivalent aggression “threat” correlated with “climbing over the partner”, a part of “on-top-of” posture when subject stands over a supine part of the partner.

In behavioral structure of SW mice pair correlations were found between “biting”, “boxing” and “circling around partner” despite the fact that the demonstration of aggression in those mice was observed on single bases only. “Circling”, in turn, correlated with defensive element “freezing”, “boxing”—with “vertical stance”. In general, aggressive elements poorly correlated with other behavioral elements since aggressive demonstrations were observed in isolated cases.

As was found, behavioral structures of TNA and SW mice had more correlations between elements of non-aggressive behavioral categories than the structure of TA mice.

3.3. Factor analysis

Factor analysis for all subjects revealed 14 factors that accounted for 71.8% of the total variance (Fig. 1). Each factor after rotation procedure accounted for nearly similar level of the variance (from 8.0% to 4.1%). Multivariate analysis of variance identified significant differences between mouse lines considering factors 2–4, 7–10 (Table 3). Factors 2, 8 and 9 showed that behavior of SW line differed from both TA and TNA mice while factors 3 and 7 appeared heavier in TA than in both SW and TNA mice. Factor 4 was different between TA and SW mice, while factor 10—between TA and TNA mice. However, these variations could be explained considering the fact that the analysis is based on high loading for the factors. For example, factor 4 is highly loaded by parameters of aggressive elements such as biting and rushing to the attack, but weight of the factor is similar in TA and TNA mice. At the same time factor 8 loaded by parameters of passive elements such as freezing, lying with sniffing, passive contact with partner and palpebral closure, was against similar in TA and TNA mice. We conclude that for this type of data management, analysis of variance is not suitable technique to analyze factor structure of the behavior.

Parameters with high loadings for main factors accounted for each mouse lines are presented in Table 4. Factor analysis for TA mice revealed that first five factors accounted for 72.7% of the total variance while factor 6 showed loadings for only one variance and was therefore not considered in the analysis. Among these factors, three principal factors were identified that accounted for 53.6% of the variance. Thus, factor 1 was related to exploratory activity such as sniffing of partner’s body, grooming of the partner, sitting with sniffing, ambivalent aggression (tail rattling) and non-aggressive contacts with the partner (passive contact with partner). Factor 2 related to non-aggressive contacts with the partner (passive contact with partner), ambivalent aggression (tail rattling, circling around partner) and consummate aggression (fighting). Factor 3 related to defense (avoidance) and self-grooming.

For TNA mice only three factors emerged clearly that accounted for 62.4% of the total variance while factors 4 and 5 were excluded because they were one variance loaded. Factor 1 was loaded highly by elements of partner exploration (sniffing of partner’s body), non-aggressive contacts with the partner (passive contact with partner, grouping together with partner, climbing under), exploration (sitting with sniffing), palpebral closure. Factor 2 was set to exploration (lying with sniffing, sitting with sniffing, sniffing of partner’s body, grooming of the partner), defense (freezing) and non-aggressive contacts with the partner (passive contact with partner) also. Parameters of passive contact with partner (MDR) and sitting with sniffing (MDR) were shared between both factors 1 and 2. Factor 3 was related to exploration (sitting with sniffing, lying with sniffing, sniffing of partner’s anogenital area), ambivalent stances (vertical stance), stretched attend posture and self-grooming.

Factor structure of SW mice consisted of three factors that accounted for 57% of the total variance while factors 4–6 were excluded because they were loaded with one variance. Factor 1 was related to exploration (sitting with sniffing, locomotion with sniffing, sniffing of partner’s anogenital area), rears and non-aggressive contacts with the partner (passive contact with partner, climbing under). Factor 2 was related to partner explo-

Table 3
Differences between mouse lines found by multivariate analysis of variance based on rotated factor analysis counted for set of all parameters

Factor	F	P	Observed power (a)	Related elements loading factor greater than 0.50	Differences (post hoc test)
Factor 2	3.125	.034	0.70	MDR_Kk; RF_Bx; RF_VS; RF_F; RF_LS; RF_B	(TA = TNA) > SW
Factor 3	4.88	.005	0.89	MDR_Ch; RF_TlRt; MDR_Av	TA > (SW = TNA)
Factor 4	3.66	.018	0.77	RF_B; MDR_RshA; MDR_B	(TA = TNA) > (SW = TNA)
Factor 7	4.00	.013	0.80	MDR_Thr; MDR_Bx; MDR_tr	TA > (SW = TNA)
Factor 8	6.50	.001	0.96	MDR_Fz; MDR_ln; MDR_Pass; RF_Pass; MDR_plp	(TA = TNA) > SW
Factor 9	9.70	.000	0.99	RF_SxSnPr; RF_SnPr; RF_ClO	(TA = TNA) > SW
Factor 10	3.64	.019	0.77	MDR_SnPr; MDR_SAP	(TA = SW) < (TNA = SW)

Note: The abbreviations of the behavioral elements are decoded in Table 1. MDR: medial duration; RF: relative frequency.

Rotated Component Matrix

	Component													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
RF_In	.889													
RF_App	.885													
MDR_LSn	.629													
RF_LSn	.582				.581									
MDR_st	.581													
RF_SAP	.557													
MDR_Kk		.811												
RF_Bx		.787												
RF_VS		.768												
RF_F		.722												
RF_LS		.647												
RF_B		.537		.519										
MDR_Ch			.698											
RF_TlRt			.599											
MDR_Av			.529											
RF_Av														
MDR_Cir														
MDR_F														
RF_R														
MDR_RshA				.844										
MDR_B				.573										
MDR_TlRt														
MDR_App														
MDR_VS					.937									
MDR_LS					.837									
RF_StSn					.796									
MDR_Grp						.798								
MDR_StSn						.650								
RF_Grp	.615					.626								
MDR_ClU						.542								
MDR_Thr							.793							
MDR_Bx							.580							
MDR_tr				.562			.565							
MDR_Fz								.830						
MDR_In								.710						
MDR_Pass								.583						
RF_Pass								.558						
MDR_plp								.545						
RF_SxSnPr									.858					
RF_SnPr									.853					
RF_ClU									.615					
MDR_ClO														
MDR_SnPr										.741				
MDR_SAP										.640				
MDR_Ev											.873			
MDR_R											-.591			
MDR_Gr											-.534			
MDR_QL												.699		
MDR_sc												-.665		
MDR_GrPr												-.501		
MDR_rt													.780	
RF_rt													.738	
MDR_SxSnPr														-.811
MDR_fd														-.621

Extraction Method: Principal Component Analysis.
Rotation Method: Equamax with Kaiser Normalization.

Fig. 1. Principal parameters loading to factors emerged by factor analysis with Equamax rotation factor analysis with Equamax rotation was done for set of behavioral parameters of TA, TNA and SW mice together. Parameters loading to factors greater than 0.5 are shown. The abbreviations of the behavioral elements are decoded in Table 1. MDR: medial duration; RF: relative frequency.

Table 4
The elements loading on factors counted for TA, TNA and SW mice independently

	TA			TNA			SW		
	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3	Factor 1	Factor 2	Factor 3
Variance explained	24.1%	16.2%	13.4%	25.3%	20.8%	16.3%	26.8%	16.3%	13.9%
Consummate aggression		F(MDR): −0.557							
Ambivalent aggression	TlRt(MDR):0.714	TlRt(RF): 0.675; Cir(MDR): 0.587							
Defense		Fz(MDR): −0.606	Av(MDR): −0.572		Fz(MDR): 0.772				
Partner exploration	SnPr(MDR): 0.673; GrPr(MDR): 0.699	SnPr(RF): 0.698		SnPr(MDR): 0.740	SnPr(RF): −0.642; GrPr(MDR): −0.609	SxSnPr(MDR): −0.642	SxSnPr(MDR): 0.514	SnPr(MDR,RF): 0.608, −0.643	
Ambivalent stances						VS(MDR): 0.588			
Locomotion							LSn(RF): −0.743		LSn(MDR): −0.648
Non-aggressive contacts with a partner	Pass(MDR): 0.814			CIU(MDR): 0.688; Pass(MDR,RF): 0.675, −0.750; Grp(MDR): 0.689			CIU(MDR): 0.618; Pass(MDR): 0.609		CIO(MDR): 0.619
Self-grooming			Gr(MDR): 0.620			Gr(MDR): 0.579		Gr(MDR): −0.616	
Rears							R(MDR,RF): 0.559,−0.725		
Sitting with sniffing	StSn(RF): −0.775			StSn(MDR): 0.682		StSn(RF): 0.784	StSn(MDR,RF): 0.782, −0.630		
Other behavior				plp(MDR): 0.548	ln(MDR): 0.673	SAP(MDR): −0.601		rt(MDR): 0.625	SAP(MDR): −0.503

Note: The value of loading is correlation of the variable with the factor. The abbreviations of the behavioral elements are decoded in Table 1. MDR: medial duration; RF: relative frequency.

ration (sniffing of partner's body), self-grooming and rotation. Factor 3 was related to locomotion with sniffing, rears, climbing over and stretched attend posture.

3.4. Discriminant function analysis

Discrimination between TA, TNA and SW mice was done successfully: 100% of original grouped cases and 100% of cross-validated grouped cases were classified correctly (with cross-validation, each case in the analysis was classified by the functions derived from all cases other than that case). The result holds true for the discriminant functions built on the factor structure of combined mouse behavior and on set of behavioral parameters. Standardized canonical discriminant function coefficients are illustrated on Fig. 2. Discriminant functions built on factor structure identified that function 1 depended significantly on coefficients for factors 3, 4 and 7, function 2 depended significantly on coefficients for factors 6, 8 and 9. Discriminant functions built on behavioral parameters identified "circling around partner", "lateral stance" and "vertical stance" as principal variables for function 1. These parameters were supplementive to discriminate TA and TNA behaviors since there was no high loading for any significant discriminant factors. In turn, parameters defined function 2 such as "sniffing of partner's body", "passive contact with partner" and "tremor" highly loaded on factors 7–9, respectively. Found divergence between factors and independent behavioral parameters means that both ways of discriminant function analysis are important and should be performed together. In our case both techniques produce similar results and showed highest level of discrimination. According to the analysis, the hypothetical function of behavioral profile is 14-component factor's functions $F1 = 3.8(\text{factor } 3) + 3.04(\text{factor } 4) + 3.11(\text{factor } 7) + \cdots + 1.6(\text{factor } 14)$ and $F2 = 0.66(\text{factor } 6) + 1.2(\text{factor } 8) + 1.35(\text{factor } (F9) + \cdots + 0.32(\text{factor } 14)$ or 18-component behavioral parameters functions $F1 = 1.43(\text{MDR\_Cir}) - 2.35(\text{MDR\_VS}) + 2.2(\text{RF\_LS}) + \cdots$ and $F2 = 0.96(\text{RF\_SnPr}) + 1.4(\text{RF\_Pass}) + 0.1(\text{RF\_tr}) + \cdots$ where MDR and RF is medial duration and relative frequency of behavioral elements. Symbols of main principal behavioral elements are presented in Table 1.

4. Discussion

With the use of analysis of variance method, the differences between TA, TNA and SW mice were identified for all behavioral categories except for medial duration of ambivalent stances and relative frequency of self-grooming. Despite the fact that most differences were found between TA and TNA mice which were more close to SW mice, defense category factor appeared to have similar value for both TA and TNA but was higher than value determined for SW mice. Also, the frequency of locomotor behaviors appeared to have similar value for TA and TNA mice. However, we cannot produce the clear conclusions based on the results obtained by the use of linear model only, since other links between behavioral parameters were not clarified. Thus, medial duration of "vertical stance" appeared as espe-

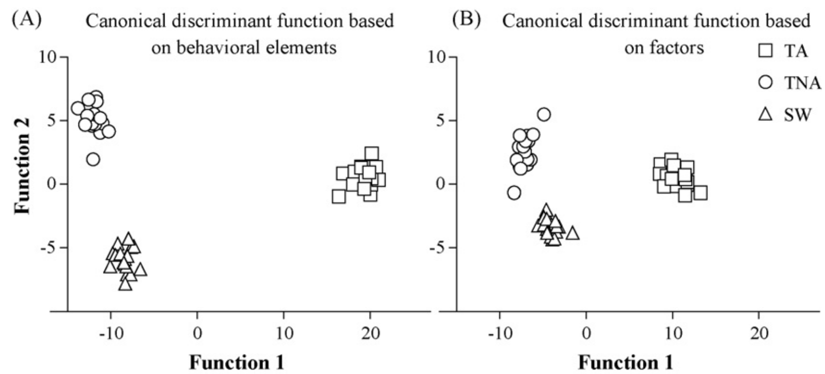

Fig. 2. Illustration of the results by discriminant function analysis based on factors counted for set of all behavioral parameters (A) or based on all parameters of behavioral elements (B). Main coefficients of discriminant functions 1 and 2 and principal elements to discriminate the mouse lines are presented in Section 3.

cially important parameter to discriminate mouse lines based on discriminant function analysis while analysis of variance failed to find significant differences between the lines. This statistical divergence emphasizes the importance of use of multi-sided statistical techniques for data management.

As it was mentioned above, the similarity between frequency of locomotor behavior and rears in both TA and TNA mice makes it difficult to evaluate TNA mouse behavior as suppressive one. However, level of risk-assessment behavior, stretched attend posture, was the highest in TNA mice, suggesting increased level of anxiety in those mice (Rodgers et al., 1997). Similar conclusion was done previously when behavior of both TA and TNA lines was observed in anxiety test battery (Nyberg et al., 2003). In context of subject–partner interaction longer duration of rears (vertical activity) found also in TNA mice might be also interpreted as risk-assessment behavior (Blanchard et al., 1998) and attempt to escape from the experimental arena.

It must be noted that the numerous significant differences found between the lines by analysis of variance were highly depended on number of animals used in the experiment. Previous findings when small number of animals was used, identified agonistic (consummate aggression and defense) differences only between TA and TNA lines (Nyberg et al., 2003). This instability of results obtained by analysis of variance method encourages the use of other statistical techniques while accessing the experimental data.

Despite the fact that medial durations of ambivalent stances were similar in TA and TNA mice, correlation analysis identified them as threatening postures for TA mice because the elements correlated with aggressive ones only while in TNA mice the stances appeared as both defensive, an attempt to keep a partner at arm's length, and threatening when it correlated with "boxing", behaviors. It seems that TNA mice were able to demonstrate aggressive behavior as well. In turn, the interpretation of the ambivalent behavior had to be based on statistical results to minimize subjective component of observation.

According to correlation analysis, the weak TNA correlations between elements of aggressive and defensive patterns emphasize the poverty of agonistic expression that might dependent on partner's behavior. Thus, the correlations between ambivalent aggression and dominant demonstration "climbing over the partner" (Kahana et al., 1997) might be interpreted as response to partner's insubordination or peaceful demonstration of domination.

Structure of aggression in SW mice was mostly based on biting–boxing observations, while in TA mice it was based on fighting–biting correlations. Also, poor relations of aggressive elements with other behavioral elements in SW mice suggest that demonstration of aggressive behavior is most likely probable. Moreover, the aggression of TA mice might be described as offensive, because defensive elements were excluded from correlation links. On the contrary, TNA and SW aggression might be classified as defensive.

Despite of the strongly pronounced aggressiveness of TA phenotype, its factor structure was not completely based on aggressive elements. Consummate aggression "fighting" loaded highly on factor 2 while factors 1 and 3 were loaded by partner and environment exploration, demonstration of aggressive intentions as well as defensive elements. Statistically, the elements loaded first factor might be described as most likely events for the behavioral structure. The numerous non-agonistic parameters high loading for factor 1 suggest "normality" of TA aggression, although these mice display it in any given environment conditions (Nyberg et al., 2004).

Factor structure of TNA mice was based on partner exploration, non-aggressive contacts with the partner and defense. In addition, a risk-assessment element "stretched attend posture" loaded highly on factor 3 was also included. It suggests that partner-induced anxiety-like condition and demonstration of defensive strategy based mostly on immobility rather than on escape strategy. In addition, "palpebral closure" loaded on factor 1 that has a resemblance to gaze-avoidance, or cut-off, protective human posture of flight behavior (Dixon, 1998), and might be also considered as a passive form of defense.

Factor structure of SW mice was not related to any agonistic parameters although the presence of risk-assessment behavior, stretched attend posture, suggests anxiety-like condition in mice (Rodgers et al., 1997). In the context, the vertical activity loaded highly on factor 1 might be discussed as attempt to escape social arena. Both, rears and active sniffing are always observed in rodents in unfamiliar situations and may represent as a risk assessment towards potentially threatening stimuli (Blanchard et al., 1998).

Thus, principal combinations of behavioral parameters loaded highly on factors identified distinctions between TA, TNA and SW mouse behavioral structures "in space" and clarified behavioral profile for each mouse line. Regrettably, quantitative comparison between lines was impossible when the factor structure was calculated for each line independently (Child, 1971). This limitation links factor analysis with sequence (Brain et al., 1985; Jones and Brain, 1985) or Discrete model (Poshivalov and Khodko, 1984; Poshivalov et al., 1988) analyses that also are suitable for qualitative analysis only. However, this cluster technique which is required to observe behavioral elements consecutively (Jones and Brain, 1985) proved to be unacceptable in our case, since some elements were recorded in parallel and cannot be build into a sequence. Proposed multimetric statistical method ignores element classification although for cluster techniques it should be done initially to interpret the cluster structure. Whereas we also classify observed elements, some of them such as "avoidance" and "circling around partner" were calculated for several categories at the same time to avoid subjectivity. Ambivalent stances (vertical and lateral) were also used instead "upright or sideways offensive" or "upright and sideways defensive" elements (Jones and Brain, 1985) that are looking too similar to discriminate between them. We believe that observed elements with the exception of consummate factors might be clarified correctly when analyzed using statistical analysis.

Clear discrimination between TA, TNA and SW mice found by discriminant function analysis based on factor structure or set of behavioral parameters proved that each mouse line has its own social profile. Hypothetical discriminant formula for mouse behavioral profile would allow to identify behavioral profile of genetically unknown subject during future studies and, therefore, mouse line affiliation.

Thus, multimetric analysis successfully identified different profile of three mouse genotypes that might be correlated with human traits despite the fact that most of the human features are represented in terms that are quite different compared to mouse behavior (Gosling and John, 1999). However, Cloninger (1987) three-dimensional model of personality might be accepted for the mice. The various combinations of dimensions such as novelty seeking, harm avoidance and reward dependence might describe temperament and character of the subject (Cloninger, 1987). The behavioral profile of TA mice shows rapid adaptive ability (Nyberg et al., 2003), aggressive, competitive, overactive, socially detached, that might be described as high novelty-seeking, low harm-avoidance and low reward dependence. According to personality cluster this combination of the dimensions suggests impulsive or opportunistic, or oppositional temperament while in light of personality disorders it indicates antisocial disorder.

TNA mice demonstrated passive defense, low aggression, lower level of locomotion and active exploration that might be construed as sensitive to social cues, rarely becoming angry, inhibited by unfamiliar situation and strangers behavioral profile. It corresponds with high reward dependence, low novelty seeking and high harm avoidance combination of personality dimensions that suggests rigid or scrupulous, or oppositional personality temperament or passive-avoidance personality disorder. Thus, multimetric statistical analysis lightened the interpretation of mouse characteristics in terms of human personality model in frame of one social test that might open new directions to model mental disorders and treatment screening.

When investigating animals, the coping style of the aggressive mice considers active manipulation while non-aggressive individuals prefer passive confrontation. The success of both coping styles depends upon the variability or stability of the environment. If aggressive style will be advantageous for predictable (stable) situations, the flexible behavior of non-aggressive individuals will be of advantage under changing conditions (Benus et al., 1991).

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