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Measuring sociability of mice using a novel 3-chamber set-up and algorithm of an automated animal behaviour analysis system

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Background:

Autism spectrum disorder is characterized by impaired sociability as one of the major symptoms in humans. The 3-chamber sociability test is a widely accepted animal behavioural assay which can be used to examine social behaviour of rodents [1,2].

Aim of the study:

Here we designed and validated a new algorithm to measure social preference of mice in an automated behavioural analysis system (LABORAS™, Metris b.v., the Netherlands) [3]. The system allows parallel automated observations therefore, time and labour consuming observational work can be replaced. Validation involved the investigation of three different mouse strains with various levels of social behaviour and the effects of drugs (3,4-methylenedioxy-methamphetamine (MDMA) and phencyclidine (PCP)). Furthermore, it was also investigated how aggression contributes to social behaviour in this assay.

Methods:

As a validation, we compared the results of the system with the scores of two independent blinded observers using parallel recorded videos. Time spent in chambers and in contact zones around the social/non-social cylinders were determined (Fig. 1). During validation we optimized contact zone radius to find the one most matching with observers' scores. Optimized contact zone radius of the set-up was chosen to be 90 mm (Fig. 2). As a target mouse DBA/2 strain was used and placed into the cylinders, except when DBA strain was measured wherein NMRI mice were used. Male mice weighing 25-40 g were obtained from the following breeders: NMRI from ToxiCoop, Hungary; C57Bl6/J and DBA/2 from Envigo (formerly Harlan), Germany/UK, and CD-1 from Charles River, Germany. Drugs and vehicle (phys. saline) were administered intraperitoneally in a volume of 10 ml/kg. Statistical analysis was performed with GraphPad Instat or GraphPad Prism (GraphPad, San Diego, USA).

Validation of the algorithm *in order to find the best matching parameters with observers' scores*

Fig. 1

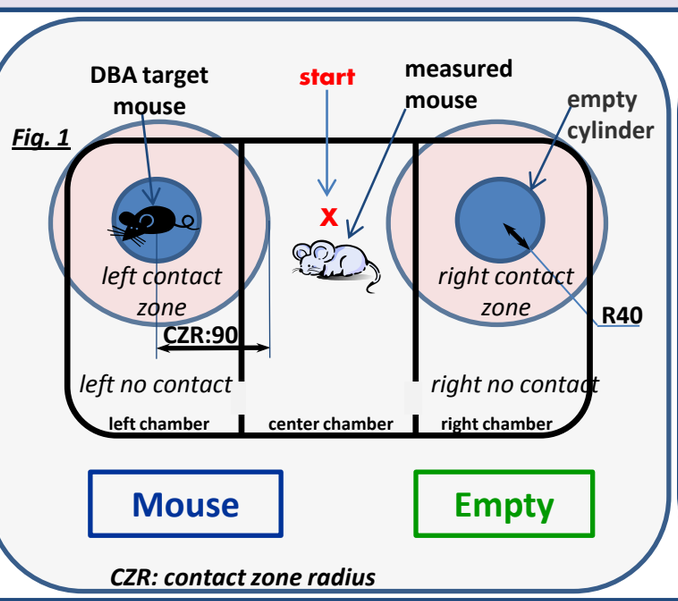
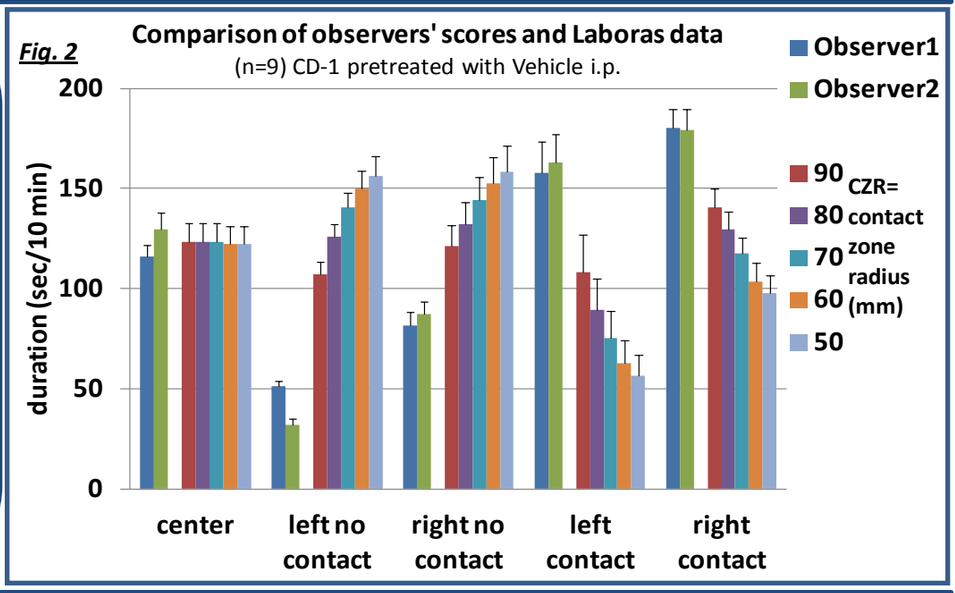


Fig. 2



Conclusion:

1. We validated and optimized a new mouse sociability algorithm of LABORAS system with the comparison of results determined by the system and the scores of two independent blinded observers.
2. We could measure alteration of sociability due to:
   - increase of time spent in contact with social cylinder or
   - decrease of avoidance and less time spent in contact with the empty one.
3. Changes in sociability caused by drugs and at the same time their effect on activity could also be measured with this new sociability set-up in mice.

Measuring sociability in different mouse strains

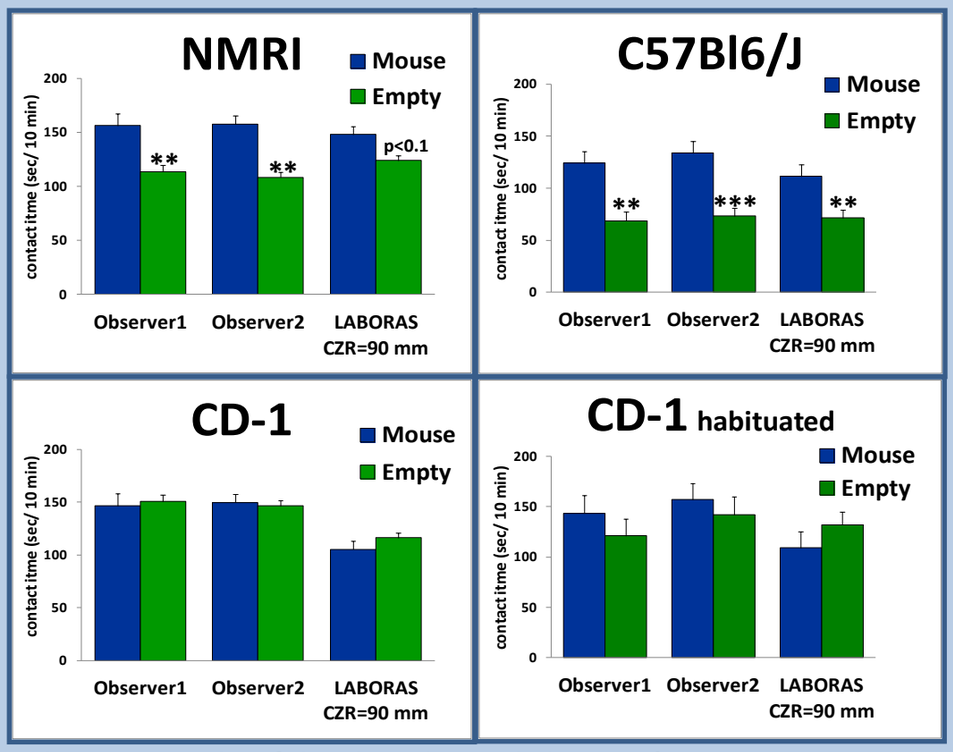


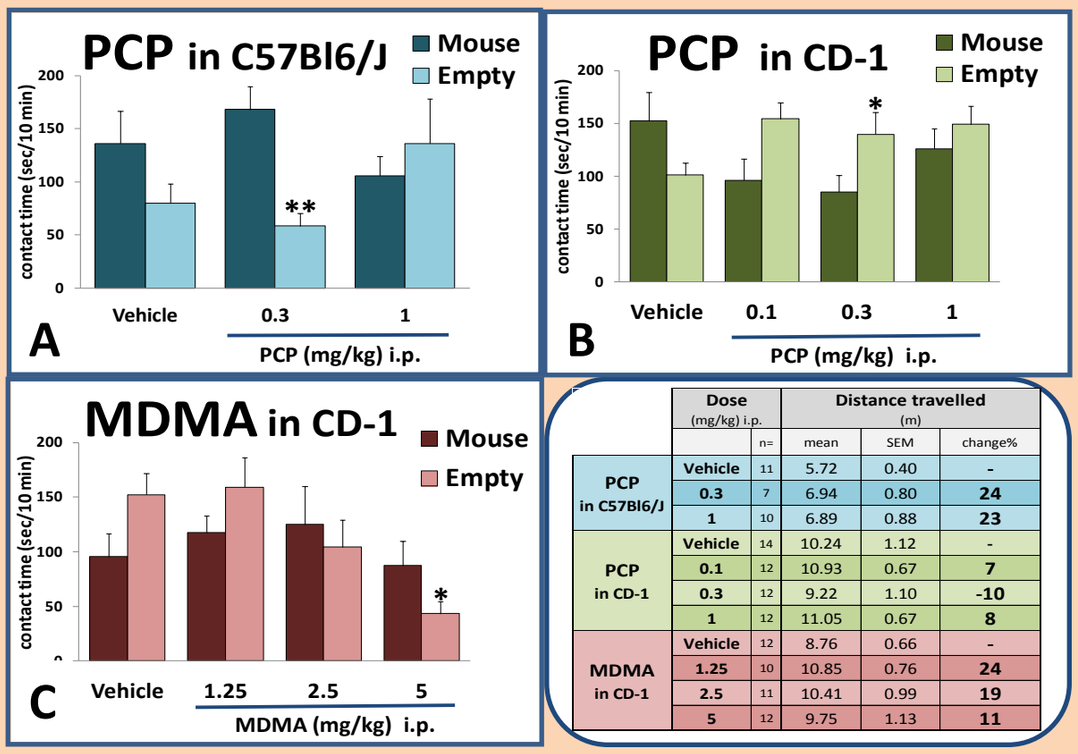
Fig.3

Comparison between results of the algorithm and observers' scores.

NMRI (A, n=28) and C57Bl/6J (B, n=16) strains showed normal sociability;
they preferred to spend more time in contact with the social cylinder compared to the empty one.
In contrast to this, CD-1 mice (C, n=44) did not spend more time with the contact of mouse; rather aggressive, often offensive behaviour could be observed. Habituation to the cage (2' in center and 5' in whole) did not modify non-social behaviour of CD-1 mice (D, n=12).

Statistical analysis was performed with paired t-test and with 2-way ANOVA with post hoc Sidak test.
*****; p<0.01, ***; p<0.001 vs Mouse contact time***

Pharmacological manipulation of social behaviour



	Dose (mg/kg) i.p.	n=	Distance travelled (m) mean	SEM	change%
PCP in C57Bl6/J	Vehicle	11	5.72	0.40	-
	0.3	7	6.94	0.80	24
	1	10	6.89	0.88	23
PCP in CD-1	Vehicle	14	10.24	1.12	-
	0.1	12	10.93	0.67	7
	0.3	12	9.22	1.10	-10
	1	12	11.05	0.67	8
MDMA in CD-1	Vehicle	12	8.76	0.66	-
	1.25	10	10.85	0.76	24
	2.5	11	10.41	0.99	19
	5	12	9.75	1.13	11

Fig. 5.

PCP deteriorated whereas MDMA ameliorated decreased social behaviour of mice.

In C57Bl6/J mice PCP at the dose of 0.3 mg/kg did not modify social preference, while 1 mg/kg dose impaired it and produced similar contact time on both sides (A). In CD-1 mice PCP (0.1-1 mg/kg) also showed this social impairment effect started at lower dose and it seemed to increase their avoidance behaviour. At the dose of 1 mg/kg the initiation of activity increasing mechanism might cause the elevation of contact time on both sides (B).

MDMA, similarly to its prosocial effect observed in neurotypical humans [4] was able to ameliorate decreased social behaviour of CD-1 mice in a dose-dependent manner via decrease of contact time with the empty cylinder (C).

Mice were 2'+5' min habituated. Statistical analysis was performed with paired t-test or 2-way ANOVA with post hoc Sidak test. *; p<0.05, **; p<0.01 vs Mouse contact time

Aggressive behaviour and sociability

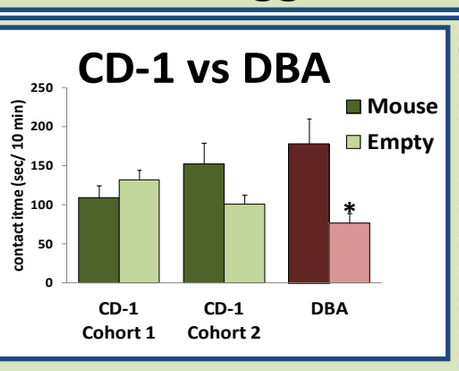


Fig.4

CD-1 mice did not prefer social contact and this was not the direct consequence of their offensive nature.

In order to distinguish antisocial and non-social behaviours, an aggressive subgroup of DBA mice was also investigated. Since this subgroup of DBA strain spent more time on the mouse side of chambers, thus the existence of aggressivity alone did not explain avoidance behaviour of the CD-1 strain.

Mice were 2'+5' habituated. CD-1 Cohort1: n=12, CD-1 Cohort2: n=14, DBA: n=8. Statistical analysis was performed with paired t-test. *; p<0.05 vs Mouse contact time

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