1 Benowitz, N. L., A. Rev. Med. *37* (1986) 21.
2 Ogle, C. W., Cho, C. H., and Wong, S. H., Experientia *41* (1985) 1140.
3 Qiu, B. S., Cho, C. H., and Ogle, C. W., Agents Actions *33* (1991) 367.
4 Yano, S., Akahane, M., and Harada, M., Jap. J. Pharmac. *28* (1978) 607.
5 Vantrappen, G., Vanderbroucke, T., Verbeke, S., and Hellemans, T., in: Pathophysiology of Peptic Ulcer, pp. 115–131. Ed. S. C. Skoryna. McGill University Press, Montréal 1963.
6 Brodie, D. A., and Kundrats, S. K., Fedn Proc. *24* (1965) 714.
7 Cho, C. H., and Ogle, C. W., Eur. J. Pharmac. *48* (1978) 97.
8 Ageel, A. M., Parmar, N. S., and Tariq, M., Life Sci. *34* (1984) 751.
9 Cho, C. H., Ogle, C. W., Wong, S. H., and Lam, S. K., Hormone Res. *25* (1987) 113.
10 Takagi, K., and Okabe, S., Eur. J. Pharmac. *10* (1970) 378.
11 Goldman, H., and Rosoff, C. B., Am. J. Path. *52* (1968) 227.
12 Watanabe, K., Chem. pharmac. Bull. *14* (1966) 101.

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Deficits in social behavior in autism and their modification by a synthetic adrenocorticotrophic hormone (4-9) analog

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Abstract. When charting the structure of the social behavior of autistic children by means of an ethologically analyzed playroom session, deficits appeared in the reciprocity of eye-contact and in the location of verbal initiatives. These deficits in social behavior were beneficially influenced by treatment with the adrenocorticotrophic hormone (4-9) analog ORG 2766.
Key words. Social behavior; neuropeptides; ethology; autism.

Many important activities that people engage in take place in social settings. The quality of social functioning and the profits of social interaction contribute to a large extent to a feeling of well-being and determine the level of adaptation of an individual. Conversely, many psychiatric disorders are characterized by disturbed social behavior and associated distress.

Autism is a chronic persisting psychiatric disorder that is preeminently marked by deficits in social interaction. In addition, autistic children are characterized by repetitive stereotyped behavior patterns and a deviant development of cognitive, language and communicative functions [1, 2]. Focussing on disturbances in social behavior, it is not so much differences in frequencies of occurrence of particular behaviors, but rather the differences in the quality of social behavior that are of interest. The quality of social behavior refers to aspects of mutuality and reciprocity and to the structure of behavior. Ethologists have developed concepts and methods which enable one to analyze qualitative aspects of behavior in quantitative terms [3, 4].

Therefore, in a first study we applied ethological methods for the observation and analysis of behavior to chart the deficits in the structure of social behavior of autistic children. All subjects were included in the study only after the nature and the consequences of the procedures had been fully explained to them and their parents, and informed consent was obtained. The concept of the structure of behavior refers to the tempero-sequential relationships of various behavior elements and to the distinction of behavior systems [3, 4]. This process of pattern detection is based, among others, on the temporal contingencies of behavior elements.

The social behavior of 14 autistic children, and by way of contrast, of 10 nonautistic retarded control children was observed in a semistructured playroom session lasting 20 min. The diagnosis of autism had been made independently by two child psychiatrists according to DSM-III-R criteria (1) on the basis of extensive diagnostic evaluations, which included a review of prior records, a parent interview, a child-psychiatric observation and a complete medical diagnostic work-up. All children met the DSM-III-R criteria for autistic disorder (299.00). Age of the autistic children was 8.7 ± 2.1 years (mean ± SD). Their IQ was 69.1 ± 25.0 (mean ± SD). Controls were nonautistic retarded children matched on age and IQ: age 8.9 ± 1.7 year, IQ 81.7 ± 12.0.

In the playroom session, the child is with an unfamiliar experimenter in a playroom. The experimenter presents the child with some tasks in a fixed sequence. The tasks in this case were consecutively a constructive task (building a gate with wooden blocks), a motor activity task (hopping), a drawing task (drawing a boat on the blackboard) and a musical task (playing a tune on a flute). The time-relationships of single behavior elements are registered by means of an event-recorder. The behavior ele-

ments of the children which are recorded include a fine-grained scoring of gaze behaviors, talk behaviors, stereotypies, reacting-to-task behavior, and nonverbal gestures. Some selectively chosen behavior elements from the experimenter are also registered, in order to examine the interaction between child and experimenter.

All scoring was done by the same observer from behind a one-way mirror. Both the intraobserver reliability (mean Kappa 0.83, the range being from 0.68 to 0.88) and the interobserver reliability (mean Kappa 0.78, the range being from 0.68 to 0.84) were quite sufficient. Temporal contingencies of behavior elements are assessed on the basis of a matrix of combination frequencies within a time frame of 5 s. For all cells of this matrix residual values were calculated, which served as a measure for the strength of the temporal contingencies. We expressed the residuals R as proportions of the frequencies expected under the null-model of independence. Thus, for each cell R is the difference between the observed and the expected frequency divided by the expected frequency. These R-values were used to compare different contingencies in one matrix as well as to compare the same contingencies in different matrices.

The comparison of the structure of social behavior between the two groups (fig. 1) revealed that gaze behaviors of child and experimenter (face C and face E) in the control group show high temporal contingencies, in contrast to the autistic group. This indicates that in the control group facing behaviors occur close together in time, pointing to the establishment of mutual eye contact. Conversely, facing behaviors by the autistic children occur temporally independently of facing by the experimenter. A second difference concerned the strong contingencies between verbal initiatives (e.g. putting a question, shifting the conversation to a new topic) and stereotypies in the autistic group. Obviously, verbal initiative by the autistics is connected strongly with stereotyped behaviors. This denotes a rigid way of making verbal initiatives, such as asking again and again for the same thing. By contrast, in the control group verbal initiative is associated with nonverbal communicative responding, such as pointing or showing, which means that the focus of attention in the conversation is being shifted to something new.

ORG 2766, a synthetic adrenocorticotrophic hormone (4-9) analog, was shown to modulate changes in social behavior elicited by environmental manipulations in rats[5]. In addition, ORG 2766 was able to counteract a decline in social attention in aging rats[6], and exerted beneficial effects on social behavior in elderly people[7].

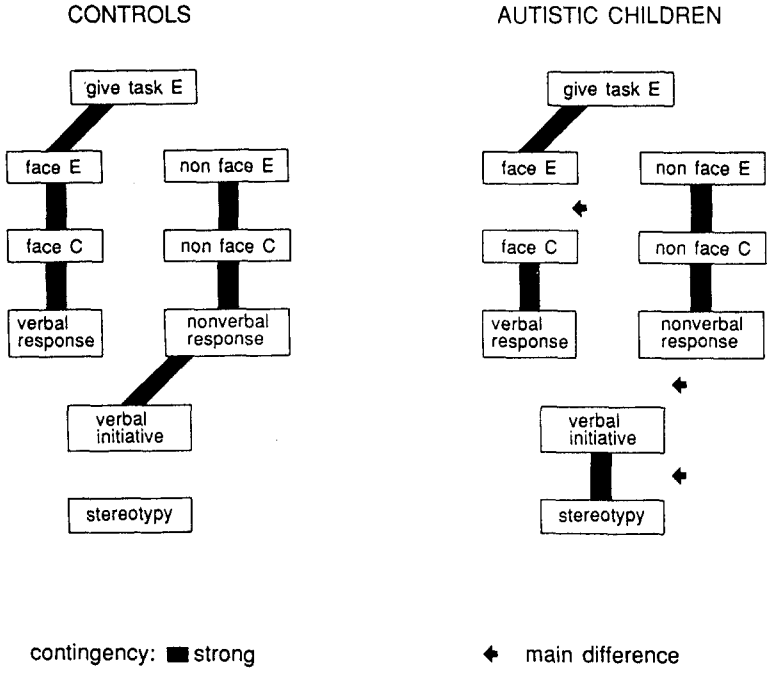

Figure 1. Pictogram of the temporal contingencies measured by the residuals (R) between behavior elements[8]. At the left the control group (N = 10), at the right the autistic group (N = 14). Strong contingencies (R > 0.40) are indicated by dense connecting lines. C: behavior of the child. E: Behavior of the experimenter.

Post hoc, main differences in contingencies between both groups were analyzed further by comparing intragroup variability of R values (mean ± SD) with the Mann-Whitney U-test: face E – face C controls 0.79 ± 0.25, autistics 0.04 ± 0.32, $p < 0.01$; non verbal response – verbal initiative controls 0.55 ± 0.21, autistics 0.29 ± 0.35, $p < 0.01$; verbal initiative – stereotypy controls 0.10 ± 0.17, autistics 0.55 ± 0.15, $p < 0.05$.

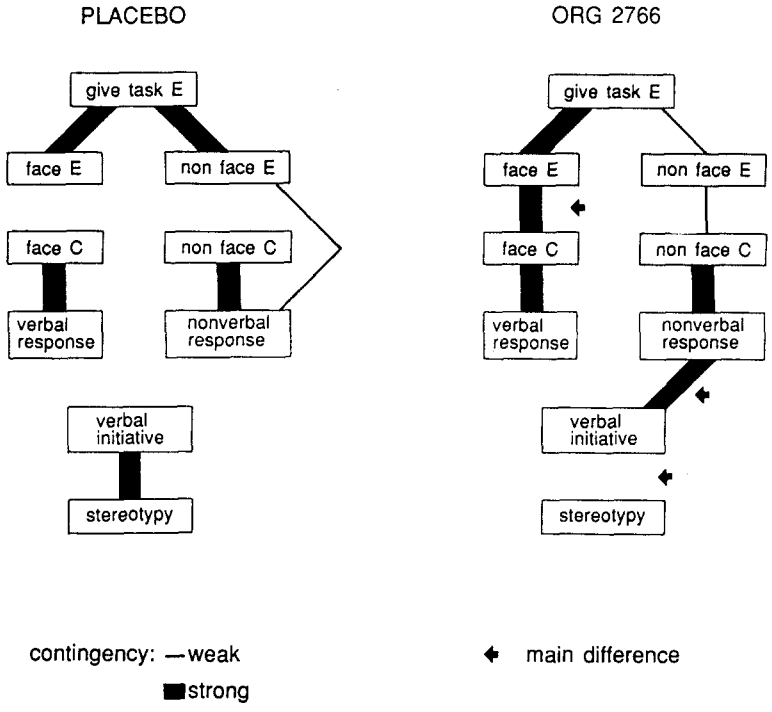

Figure 2. Same as fig. 1, but now at the left for the placebo condition and at the right for the ORG 2766 condition for a group of 20 autistic children.
Predicted differences in contingencies between both groups were tested with the Wilcoxon Matched-Pairs Rank-Sum W-test. We present the R values (mean ± SD) for both groups and the p values of the tests: E – face C placebo 0.11 ± 0.30, ORG 2766 0.75 ± 0.38, $p < 0.05$; verbal initiative – stereotypy placebo 0.48 ± 0.23, ORG 2766 0.02 ± 0.16, $p < 0.05$; non verbal response – verbal initiative placebo 0.23 ± 0.18, ORG 2766 0.45 ± 0.20, $p = 0.08$.

Furthermore, ORG 2766 beneficially influenced attentional and motivational processes in animal and human studies[8,9]. There is evidence that in autism information processing is defective[10–12].

On the basis of the results of the first exploratory study and the known effects of ORG 2766, we put forward the hypothesis that treatment of the autistics with ORG 2766 would result in a) stronger temporal contingencies between face E and face C, and also between nonverbal response and verbal initiative, and b) a weaker contingency between verbal initiative and stereotypy. To test these predictions, in a second study ORG 2766 (40 mg per child per day during 8 weeks) or placebo was orally administered to 20 autistic children (age 10.3 ± 2.1 year, IQ 62.7 ± 19.9) in a double-blind cross-over study. The influence of ORG 2766 on the structure of social behavior was examined by an ethological analysis of a playroom procedure as described above. The structure of behavior at the end of the ORG 2766 treatment was compared with that after the placebo treatment.

It appeared (fig. 2) that ORG 2766 treatment was indeed associated with shifts in the structure of social behavior, affecting in particular the temporal contingencies which were the characteristic differences between autistics and controls. Changes included stronger temporal contingencies between the gaze behaviors of child and experimenter after ORG 2766 than after placebo, which implies an improvement of eye-contact. Furthermore, it was found that verbal initiative was disconnected from stereotypies after ORG 2766, and connected to nonverbal gestures. Two of the three predictions were borne out. The third predicted change in contingencies was in the right direction, but not statistically significant. In addition, clinical ratings were obtained independently from the parents and the teachers, using the Aberrant Behavior Checklist[13], and from a child psychiatrist and a psychologist on the Clinical Global Impressions Scale[14]. These rating scores substantiated an effect of ORG 2766 treatment, mainly as a lessening of social withdrawal behavior at home.

These findings show that ethological methods can be used fruitfully in the analysis and description of qualitative aspects of social behavior in a psychiatric context. The results also indicate that specific disturbances in social behavior in man may be beneficially influenced by ORG 2766. These findings may contribute to our understanding of the neurobiological basis of social behavior in man, and of disturbances in behavior.

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1 American Psychiatric Association, DSM-III-R. Washington, APA 1987.
2 Cohen, D. J., and Donnellan, A. M., (Eds), Handbook of Autism and Developmental Disorders, New York, Wiley & Sons 1987.

3 van Hooff, J. A. R. A. M., in: Handbook of Methods in Nonverbal Behavior Research, p. 362. Eds K. R. Scherer and P. Ekman. Cambridge, Cambridge University Press 1982.
4 van Engeland, H., Bodnar, F. A., and Bolhuis, G., J. Child Psychol. Psychiat. *26* (1985) 879.
5 Niesink, R. J. M., and van Ree, J. M., Science *221* (1983) 960.
6 Spruyt, B. M., Neurobiol. Aging *13* (1991) 153.
7 Sandman, C. A., Walker, B. B., and Lawton, C. A., Peptides *1* (1980) 109.
8 De Wied, D., and Jolles, J., Physiol. Rev. *62* (1982) 976.
9 Pigache, R. M., and Rigter, H., Front. Horm. Res. *8* (1981) 193.
10 Courchesne, E., Lincoln, A. L., Kilman, B. A., and Galambos, R., J. Aut. Dev. Dis., *15* (1985) 55.
11 van Engeland, H., Roelofs, J. W., Verbaten, M. N., and Slangen, J. L., Psychiat. Res. *38* (1991) 27.
12 Verbaten, M. N., Roelofs, J. W., van Engeland, H., Kenemans, J. L., and Slangen, J. L., J. Aut. Dev. Dis. *21* (1991) 449.
13 Aman, M. G., Singh, N. N., Stewart, A. W., and Field, C. J., Am. J. Mental Defic. *89* (1985) 485 and *89* (1985) 492.
14 Psychopharmacology Bulletin, 21 (1985).

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Fusion between a myogenic cell in the satellite cell position and undamaged adult myofibre segments

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Abstract. In this report we demonstrate for the first time that differentiating myogenic cells, geographically located between the plasmalemma and external lamina of myofibres in the satellite cell position[3], can fuse directly with the plasmalemma of undamaged segments of mature myofibres.
Key words. Fusion; electron microscopy; myogenesis; skeletal muscle; in vivo.

Materials and methods

The mid region of the tibialis anterior of a mature (8-week-old) female SJL/J mouse was injured transversely by superficial application of Karnovsky's fixative[1]. Four days after injury mice under halothane anaesthesia were perfused with 2.5% glutaraldehyde (in 0.05 M cacodylate buffer pH 7.4) via the left ventricle and muscle samples were removed and placed in fresh fixative for 24 h. They were then postfixed in 1% OsO_4, dehydrated in ethanol and embedded (after critical orientation) in araldite. The blocks were then sectioned with an LKB ultramicrotome and 50-nm sections were mounted on thin bar 200-mesh copper grids, stained in a lead citrate solution and examined in a Philips 410LS transmission electron microscope at an accelerating voltage of 80 kV.

Results

In regenerating skeletal muscle of adult mice examined 4 days after chemical injury[1] numerous myogenic cells including activated satellite cells, myoblasts and myotubes were observed in the space between the plasmalemma and the external lamina of injured myofibres at a substantial distance (> 0.5 mm) from the injury site. Cytoplasmic continuity which is indicative of fusion was observed between a myogenic cell and a closely opposed normal segment of a myofibre (fig. 1). Serial sectioning demonstrated multiple cytoplasmic confluence along the closely apposed membranes of these two cells (figs 2a and 2b). The myogenic cell beneath the external lamina contained both thick (myosin) and thin (actin) filaments with evidence of sarcomeric organisation (fig. 3). This differentiating cell could have been a myoblast, or due to its

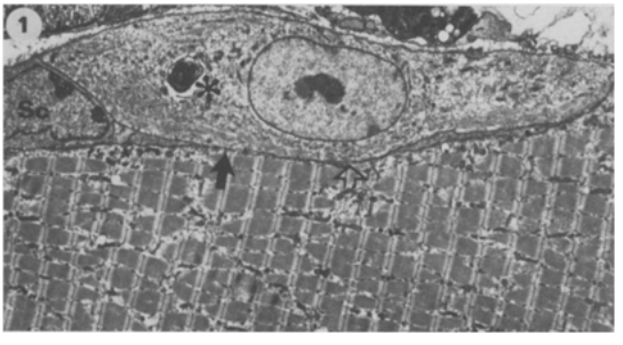

Figure 1. A myogenic cell located between the external lamina and plasmalemma of a myofibre (i.e. in the satellite cell position). Several areas of cytoplasmic confluence (see figs 2a,b,c) between these two apposed cells are marked (open and closed arrows) and a myelinoid body (asterisk) is present in the cytoplasm of this cell. An activated satellite cell (Sc) is also noted beneath the external lamina and plasmalemma of the myofibre. × 2100.

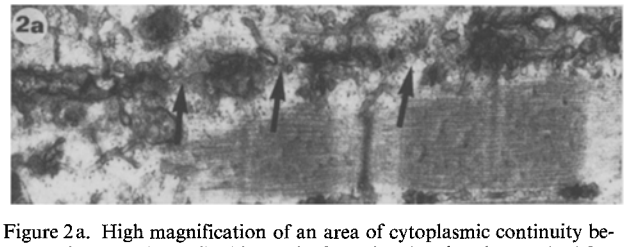

Figure 2a. High magnification of an area of cytoplasmic continuity between the apposing cells (shown in fig. 1 by the closed arrow). After extensive tilting and rotating in a eucentric goniometer, cytoplasmic confluence indicative of fusion can be observed between the two cells at several sites (arrows). × 18,840.