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Research report

Scopolamine-induced deficits in social memory in mice: Reversal by donepezil

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ABSTRACT

Deficits in social behaviour is a characteristic of numerous mental disorders including autism, schizophrenia, depression and Alzheimer's disease. For the assessment of pharmacological and genetic experimental disease models, conventional social interaction tasks bear the uncertainty that any drug-induced abnormality of the investigator may feed back to the drug-free companion modifying its reactions. A considerable technical improvement was recently reported by Moy et al. [Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson T, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behaviours in mice. Genes Brain Behav 2004;3:287–302] in which the drug free partner is confined to a small cage and social contacts of the investigator are recorded uncontaminated of any social reactions of the stranger. Using this novel behavioural paradigm, we here show in C57Bl/6 female mice that sociability (social interaction with a stranger mouse) is not impaired after administration of the anxiolytic diazepam (0.1–1 mg/kg) or the muscarinic antagonist scopolamine hydrobromide (0.1–1 mg/kg). However, social memory tested after a short time interval was impaired by both drugs in a dose-dependent manner (diazepam: ≥0.5 mg/kg; scopolamine: ≥0.3 mg/kg). The scopolamine-induced short-term memory deficit was reversed to normal by the choline esterase inhibitor donepezil (1 mg/kg).

Given this dependence of social recognition on the cholinergic system, combined with the clinical observation of reduced social contacts in dementia patients, sociability may offer a novel endpoint biomarker with translational value in experimental models of cognitive dysfunction.

1. Introduction

Impaired aspects of social behaviour are core symptoms of schizophrenia [47] and autism spectrum disorders and include inappropriate social interactions, reduced social communication and resistance to change (for review, see [39,48,56]). While some aspects common to the above diseases (especially verbal and language deficits) are impossible to mimic in rodent models with predictive validity, mice are social animals with a rich repertoire of non-verbal social interactions and social communications [27,30], which are readily amenable to quantitative experimental analysis ([14] for review). Sociability, defined as the tendency to seek social contact and interaction, and its biological basis are relatively unexplored in rodents; yet, its malfunction in a wide range of mental disorders make it an important aspect of the diseases. Understanding the genetics of social behaviour as a novel and powerful tool with considerable value for translational medicine [17] may aid in the development of novel biomarkers and therapeutic targets. Towards this end, recent progress has highlighted the importance of the following genes in rodent social behaviour: genes for neuropeptides such as oxytocin, vasopressin and estrogen and their receptors [4,11,18], the tumor suppressor PTEN [33], dopamine transporters [53], dishevelled proteins as necessary components of the Wnt cell polarity developmental signal in all cells [34,35], the Fmr1 gene leading to Fragile X syndrome [38,58], and the Mecp2 gene with crucial roles in DNA methylation and histone deacetylation [40].

Although this approach has yielded significant progress in animal models of autism, a more comprehensive understanding of social behaviour may also be relevant for other disorders with anxiety and memory related endophenotypes. This could be achieved by assessment of transmitter and receptor systems involved in social behaviour by means of pharmacological studies and eventually through evaluation of genetically modified models.

Some forms of social interaction are stressful and lead to suppression of typical exploratory responses. This appears to be particularly so when the test setting is exposed to bright light, an aversive stimulus for many rodents [18]. Under such circumstances, anxiolytic effects of drugs such as diazepam can readily be revealed by increased social activity at doses devoid of side effects on locomotion [20,21]. Here, we applied a novel sociability

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test recently developed by the group of Crawley and co-workers [42,43], in which one adult stranger is isolated in a wire-mesh cage in one part of a 3-compartment chamber. The test assesses exploratory activity towards the stranger without aversive stimuli. It is conceivable that anxiolytic drugs may not act under such circumstances and we therefore explored effects of diazepam on social interaction in this model. Whether an increase in social activity also transfers to enhanced social memory remains unexplored. While experimental conditions can be developed, in which diazepam supports memory [52], stimulation of benzodiazepine receptor binding sites are widely considered detrimental for memory formation [1,31] so much so that enhanced recognition memory will impinge negatively on later learning and impair behavioural flexibility. Indeed, we here report a dose-dependent increase in sociability after diazepam treatment, which prevented short-term social recognition memory to develop.

We have recently devoted particular interest to the cholinergic system which plays an important role in spatial and non-spatial memory formation [54]. The cholinergic system also participates in social recognition and olfactory memory [63]. In particular, systemically administered scopolamine blocked muscarinic receptors and impaired social habituation and memory in a dose-dependent manner in mice. By contrast, inhibitors of the acetylcholine esterase (physostigmine and galanthamine) reversed such deficits or enhanced social habituation [64] and ultrasonic vocalization [16] corroborating earlier work on similar compounds such as arecoline, physostigmine and tacrine [28,46,65]. In these studies, drugs were administered immediately post-training in rats providing compelling evidence that prolongation of cholinergic activity may aid the consolidation of long-term social recognition memory. When scopolamine was administered pre-training, social habituation was impaired [64] suggesting that activation of muscarinic receptors is also crucial for social learning in mice.

This situation is reminiscent of patients suffering from dementia with fronto-temporal pathology or of the Alzheimer's type, in which a reduction of cholinergic tone and reduced activation of muscarinic receptors is a consistent hallmark pathology (for review, see [62]). Moreover, people with extensive social networks (many friends and active community dwellers) are at reduced risk of cognitive impairment in old age [22,25,29,63]. In line with this observation, social disengagement is a risk factor for cognitive decline [10,23]. In clinical trials on mild and moderate dementia patients, social interactions are not a global clinical measure readily accessible to physicians as more emphasis is placed on improvements in memory and cognition at regular study visits [51]. Social behaviour can, however, become a primary outcome measure when carers are incorporated in assessment of drug effects and recent expansion of such studies confirmed improvements in social interaction in drug-trials using donepezil [7,51]. Still unexplored are the reasons for the impairment in social interaction in dementia patients; it is conceivable in this context that uncertainty of familiarity judgement owing to memory decline is a major contributing factor. Here, we explored the possibility that muscarinic receptor blockade may compromise social interaction and/or social recognition and whether this deficit is reversed/attenuated by the US Food and Drug Administration-approved anti-Alzheimer drug donepezil. We report a selective cholinergic role in social memory, but not basic sociability.

2. Materials and methods

2.1. Subjects

Free feeding, group housed C57BL/6J (17–24 g) mice (Harlan, UK) were used in these experiments. These were 7–12-week-old female mice with group sizes of $n = 9$–12 and colony-housed in groups of 5–10 with *ad libitum* access to a commercial maintenance diet and water. Mice were kept in a controlled holding environment (temperature 20–21 °C, 60–65% relative humidity) with a 12-h day–night cycle (lights on at 07:00). Tests took place during the light phase of the cycle. Animals were individually housed during experimental periods in accordance with the Federation of European Laboratory Animal Science Associations (FELASA) guidelines and in line with the UK Home Office regulations outlined in the Animals (Scientific Procedures) Act 1986.

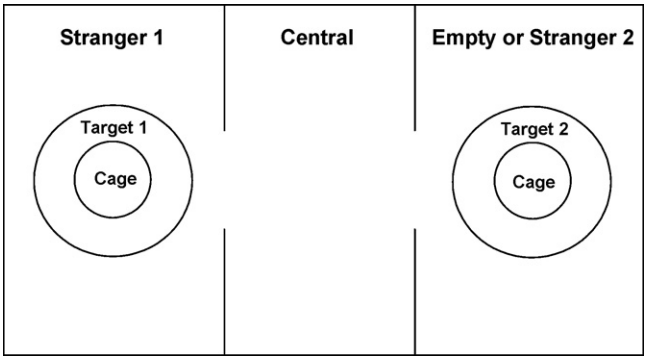

Fig. 1. Schematic view of the social interaction chamber. The white Perspex box was subdivided into a central and two side compartments; stranger 1 was placed on the left/right (counterbalanced design) side; the opposite compartment was empty (during sociability testing) or contained stranger 2 (during social recognition memory). Confinement cages made of clear perspex with holes to allow visual, olfactory and somatosensory contact were placed in the centre of each compartment; movement of the test mouse was video-recorded and stored online using Ethovision (Noldus, Wageningen, Netherlands). A direct readout for social contacts was obtained by analysis of time spent in the target zone in the immediate vicinity of the strangers.

2.2. Drugs

Animals were randomly divided into groups. Scopolamine hydrobromide was obtained from Tocris (Bristol, UK.) and diazepam was obtained from Sigma–Aldrich (UK). Diazepam was diluted in 0.5% of Tween-80, scopolamine and donepezil were diluted in saline. Diazepam and scopolamine were administered through intraperitoneal injection (10 ml/kg) 30 min prior to social interaction testing. Donepezil hydrochloride (Shanghai Honghao Chemicals, China) was administered subcutaneously (s.c.) into the dorsal neck region (10 ml/kg). For group sizes, see figure legends.

2.3. Apparatus

The social testing apparatus was adopted from Crawley and co-workers [42,43] and consisted of a three-compartment white Perspex box (Fig. 1) and each compartment was 20 cm × 42 cm × 22 cm (length/width/height). Dividing walls were made from clear Perspex, with small circular apertures (8 cm in diameter and equipped with sliding doors) enabling free access to each chamber. The two outer compartments contained small plastic cages with numerous small mesh-like holes to which stranger mice were confined for social interaction. The centre compartment was free of objects. An overhead CCTV camera recorded the activity which was stored on video tape and online using Ethovision (Noldus, Wageningen, Holland) software.

2.4. Behavioural procedures

2.4.1. Habituation

The test mice were placed in the central compartment of the box and overall activity in the arena was automatically recorded using Ethovision during a 5-min habituation period. All compartments were empty at this stage and animals allowed to freely explore the novel box.

2.4.2. Sociability test

The test animal was placed in the central compartment with apertures closed to allow exploration of the central chamber for 5 min. After this habituation period, an unfamiliar C57Bl/6J female (stranger 1) was placed inside the small Perspex cage in one of the side compartments (randomly selected and counterbalanced for each group). An identical empty Perspex cage was placed in the opposite compartment termed empty. Then, apertures were opened for the test subject to freely explore all three compartments of the apparatus over a 5-min test session. Measures taken were time spent in the compartment containing the unfamiliar mouse (stranger 1) versus the one containing the empty cage on the opposite side of the apparatus. In addition, time spent in the immediate vicinity (3 cm) of the cages (stranger 1 or empty) was calculated as direct contacts.

2.4.3. Social memory test

This is equivalent to the social novelty test by [42]. After sociability testing, animals were confined to the central compartment again and a novel stranger was introduced to the 'empty side' of the box. The design was random so stranger 1

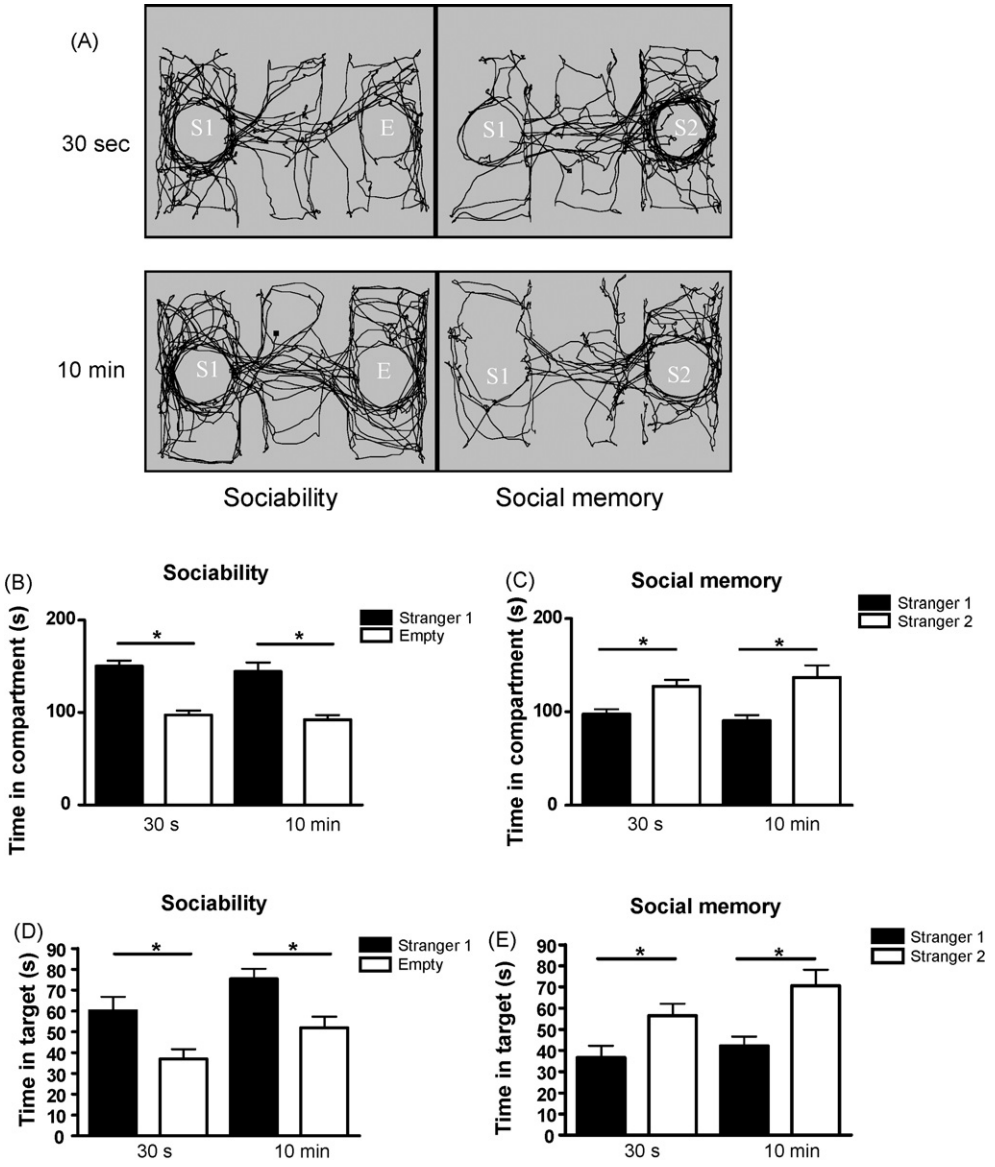

Fig. 2. Sociability and effects of time intervals on social memory in C57BL/6J mice. (A) Representative exploration paths of C57Bl/6 female mice during social exploration with stranger 1 (S1) and empty cylinder (E) in the left compartment (left column entitled sociability) and during social recognition memory with stranger 1 (S1) and 2 (S2, right column) recorded after 30 s (top) or 10 min (bottom) intervals. Group data confirm a spatial bias for the compartment containing stranger 1 (B: time in whole compartment; D: time in target area indexing social contacts) in both groups during the social interaction test. Social memory conducted 30 s or 10 min following sociability test, by contrast, revealed a strong bias for stranger 2 as measured for the whole compartment (C) or the target zone (E). Means + SEM. $N = 9–18$. Asterisks denote significance $p < 0.05$; paired t-test, two-tailed.

could remain in its place or could shift to the opposite side. A 5-min test to quantify social memory began 30 s after sociability test again recording activity in the respective compartments and the social contact zone. We also implemented a longer delay between sociability and the retention test for up to 10 min. The 3-chamber box was cleaned with soapy water and disinfected with 70% ethanol between subjects.

2.5. Statistical analysis

Data are displayed as means + SEM. Compartmental distribution was assessed using one-way analysis of variance with repeated measures followed by post hoc Student's t-test to elucidate the source of reliability. Based on the null hypothesis exploration time between stranger 1 containing compartment/contact zone and empty/stranger 2 containing compartment/contact zone should be reliably different at the level of 5%.

3. Results

3.1. Sociability and social memory in C57BL/6J mice

Mice were run in conjunction with the following treatment groups in randomized order and exposed to vehicle (Tween-80 or saline—no difference was obtained, not shown) 30 min prior to sociability testing. During the sociability test, C57Bl/6J female resident mice showed a strong preference for the compartment containing stranger 1 (Fig. 2A and B) over the empty compartment and the centre. This behaviour was noted in both groups assigned to 30 s and 10 min delays (two-tailed paired t-test for 30 s group: $t = 5.5$; df = 17; $p < 0.0001$; for 10 min group: $t = 4$, df = 8, $p = 0.004$). Moreover, resident mice expressed a strong bias for the target area surrounding stranger 1 (Fig. 2A and D) confirmed by a high number of direct social contacts (30 s group: $t = 3.3$; df = 17; $p < 0.004$; 10 min group: $t = 2.8$, df = 8, $p = 0.025$), and animals widely neglected the centre. Some exploration also occurred in the empty compartment amounting to about 30% of total time. When tested for social memory, we observed the predicted bias for the compartment containing stranger 2 (Fig. 2A and C; 30 s: $t = 2.6$; df = 17; $p < 0.017$; 10 min group: $t = 2.6$, df = 8, $p = 0.03$) as well as higher amounts of direct contacts with stranger 2 than with stranger 1 (Fig. 2A and E; 30 s: $t = 2.5$; df = 17; $p < 0.023$; 10 min group: $t = 3.3$, df = 8, $p = 0.011$).

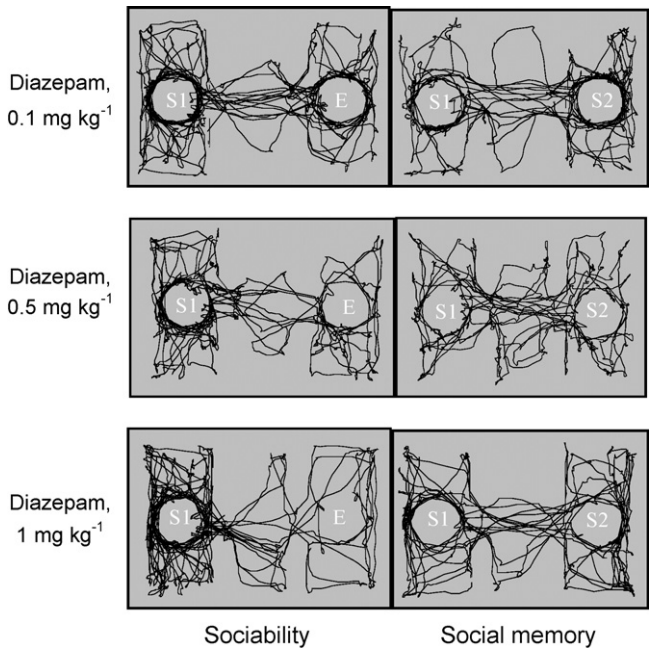

Fig. 3. Representative traces of female mice during sociability (left) and social recognition memory testing (right) when treated with different doses of diazepam. Despite a bias for the compartment and target zone containing stranger 1 (S1 and empty (E) confinements are indicated) during sociability, there was only social memory in the diazepam 0.1 mg/kg group, but not when higher doses were administered (locations of stranger 1 (S1) and 2 (S2) are marked). See Fig. 4 for group summaries.

This social recognition memory was independent of the time interval explored here and was obvious at both 30 s and 10 min delays between sociability and social memory test. In the following, we thus employed a 30-s delay only.

3.2. Sociability and social memory in diazepam-treated mice

Representative traces of exploration paths for the different doses of diazepam are displayed in Fig. 3. Although there was a clear bias towards exploring the left compartment containing stranger 1 in all treatment groups, spatial memory was only observed for the lowest dose of diazepam (0.1 mg/kg). This impression was confirmed by the pooled analyses for the groups. Preferred social interaction was obvious for all treatment groups on both the time spent in the stranger 1 containing compartment (Fig. 4A) and the respective direct contacts (Fig. 4C). The differences between stranger 1 and empty all proved reliable in each group (total time compartment: t's > 4, p's < 0.004; target time: t's > 2.7, p's < 0.025). Drug treatment also enhanced the time spent with stranger 1 and one-way analysis of variance confirmed a reliable difference ($F(3,35) = 19.7$, $p < 0.0001$) and significantly increased amount of time between vehicle control and diazepam >0.01 mg/kg (q's > 4.1, $p < 0.01$, Dunnetts test). The same significances were obtained for the time in target zone (direct vicinity) suggesting that diazepam increased the time spent with the stranger mouse, possibly due to a reduction in anxiety.

For social memory (Fig. 4B and D), we observed a significantly higher amount of time spent in the compartment containing stranger 2 for the vehicle ($t = 2.5$; df = 8; $p = 0.03$) and 0.1 mg diazepam group ($t = 4.4$; df = 10; $p = 0.001$), but not for higher doses of diazepam (t's < 1) when animals spent equal amounts with each stranger indicating no preference for social novelty (i.e. no social memory). Interestingly, this was due to an increase in time spent with stranger 1 ($F(3,35) = 7.7$; $p = 0.0005$) and not because resident mice spent less time in the compartment containing the novel stranger 2 ($F < 1$).

Overall, diazepam induced an increase in locomotion (Fig. 4E) during social interaction ($F(3,35) = 3.1$; $p = 0.04$) and social memory ($F(3,35) = 5.2$; $p = 0.005$), all drug groups differed from vehicle (all t's > 2, p's < 0.05). Despite such activity-promoting effects of diazepam, mean velocity did not differ from controls (F's < 2.2; $p > 0.1$ for sociability and social memory calculated separately).

3.3. Scopolamine had no effect on sociability but impaired social memory in mice

Overall, scopolamine-treatment did not affect sociability. Representative traces of animals in the different drug groups (Fig. 5) confirm a spatial bias towards the compartment containing stranger 1 and frequent direct contacts are obvious in the zone surrounding the confinement (left part of the chamber). These impressions were confirmed by group data analysis and statistical evaluation. All treatment groups preferred the compartment containing stranger 1 (Fig. 6A) over the empty compartment (all within-group comparisons: t's > 2.6, p's < 0.02); no statistical difference was found in time spent with their companion ($F < 1$). Similarly, all drug groups had significantly more direct contacts with stranger 1 than with the empty box (Fig. 6C; all within-group comparisons: t's > 2.7, p's < 0.017) and 0.3 and 1 mg/kg scopolamine groups spent reliably more time with stranger 1 than lower doses/control groups (ANOVA: $F(3,56) = 5.7$; $p = 0.017$; t's > 1.7; p's < 0.05, t-test, one-tailed).

As for social memory assessed after a short 30 s interval, only vehicle and 0.1 mg/kg scopolamine treated mice showed preference for the novel stranger 2 over stranger 1 (Fig. 6B; within-group comparison t's > 2.6, $p < 0.02$), but higher doses of scopolamine blocked this memory (t's < 1.2). Identical significance levels were obtained for the measure of direct contacts in the target zone (Fig. 6D) confirming that high doses of scopolamine interfered with social memory, but not social interaction.

In addition, scopolamine treatment caused an overall increase in ambulatory locomotion (Fig. 6E) relative to controls in both sociability and social memory tests (ANOVAs for each phase: F's > 5.8, p's < 0.002 followed by unpaired t-tests and t's > 2; $p < 0.05$ except comparison between vehicle and 1 mg/kg scopolamine during sociability, $t < 1$). This confirms an overall increased activity level in the scopolamine groups and is corroborated by the same significances obtained for velocity during the two test phases (Fig. 6F).

3.4. Scopolamine-induced social recognition memory deficit is reversed by donepezil

Clearly, scopolamine treatment caused a deficit in social recognition. We therefore reasoned that reversal of this deficit might be attained by co-administration of the acetylcholine esterase inhibitor donepezil (1.0 mg/kg) administered s.c. 1 min after scopolamine injection (0.3 mg/kg was selected since it induced amnesia). Drug effects are presented as exploration paths in Fig. 7 and indicate that both drug groups had normal sociability and spent most of the time interacting with stranger 1, but only the scopolamine + donepezil group presented with social recognition memory.

Pooled group data confirmed this impression for sociability and time spent in the compartment (Fig. 8A) and the respective target zone (Fig. 8C). Both treatment groups spent reliably more time with stranger 1 than in the empty compartment (t's > 2.8, p's < 0.02) or in the target zone with direct contacts (t's > 5.2; p's < 0.0001; paired t-test, two-tailed). While scopolamine treatment blocked social recognition memory for both parameters (Fig. 8B and D, t's < 1), co-administration of donepezil reversed these deficits and mice spent reliably more time with stranger 2 (t's > 2.7; p's < 0.02). Drug

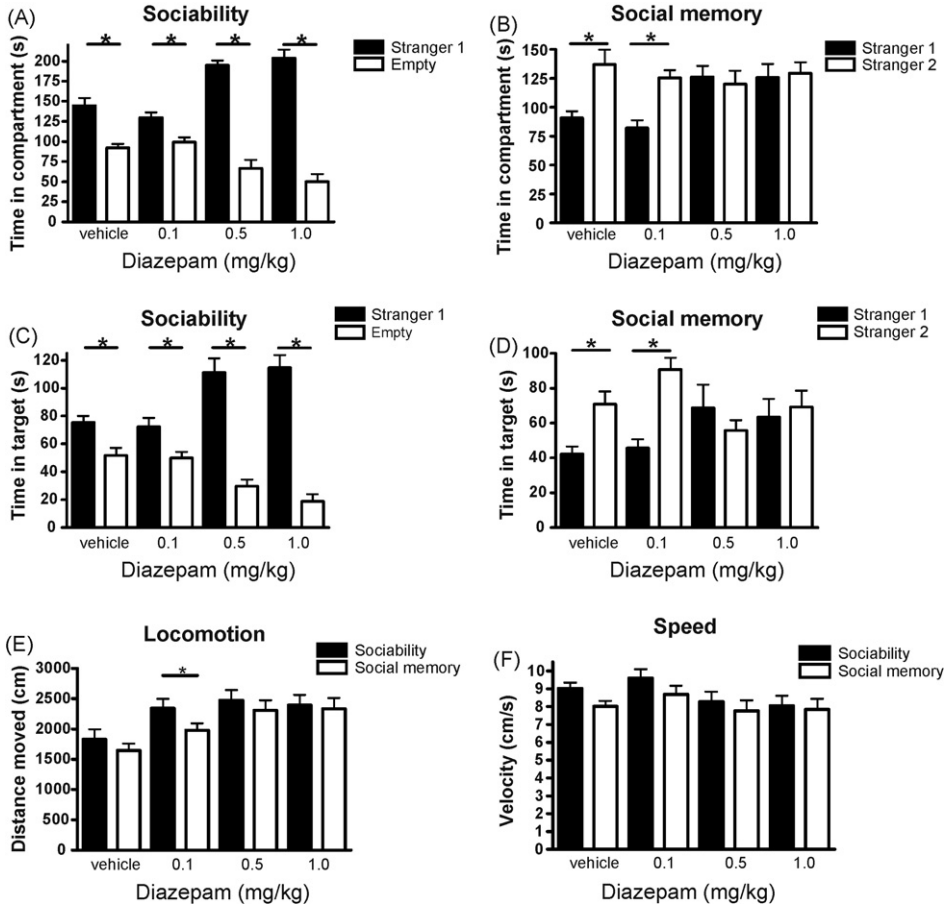

Fig. 4. Effects of diazepam on sociability and social memory in mice. Diazepam was administered i.p. in various doses 30 min prior to the sociability test. Social interaction was recorded during sociability as time in compartment containing stranger 1 (A) and as direct contacts with the confineds Stranger in the target area (C). Both parameters confirmed a bias for the compartment containing stranger 1 in all drug groups; and this time increased in a dose-dependent manner. The same parameters were observed during social memory testing (B = time in compartment containing stranger 2; D = time in target zone as direct contacts with stranger 2) after a 30 s time-interval. A bias for stranger 2 (novelty) was obvious in both vehicle and 0.1 mg/kg diazepam groups, but not at higher drug doses. (E) Overall ambulatory activity was increased due to drug treatment while velocity (F) did not differ between groups. Means + SEM for $n = 8–11$. Asterisks denote significance $p < 0.05$; paired t-test, two-tailed.

groups did not differ with respect to ambulatory activity (Fig. 8E) and movement velocity (Fig. 8F) in any phase of testing.

4. Discussion

Social interaction is a fundamental characteristic of social organisms and most likely modulated by endogenous, genetic and environmental factors. Despite its assessment in numerous animal model systems, relatively little is known about its biological and neural mechanisms. Deficits in social interaction are defined endophenotypes of several psychiatric disorders and a core feature of autism spectrum disorders (see [15,24,41] for reviews). However, there is now strong evidence that impairment in social interaction is also an intermediate phenotype or endophenotype of AD, although the underlying mechanisms and causes remain elusive. Not surprisingly, a 12-week multi-centre and a phase IV 12 months trial have independently confirmed that anti-Alzheimer treatment with donepezil (Aricept©) enhances patients social interaction, engagement and interest as attested by carers [7,51].

Here we assessed social interaction in mice in a three-compartment apparatus and determined sociability, i.e. social interaction of a resident mouse with a stranger confined to a small box in one compartment, and after an interval evaluated social recognition memory, i.e. discrimination of a familiar stranger 1 from a novel stranger 2. This discrimination is typically observed in mice [41] and rats (Deiana and Riedel, unpublished observations) and we present evidence that female C57Bl/6J mice can readily recall a familiar conspecific for an interval of 30 s to 10 min. In spatial learning paradigms, this time-interval is in the range of short-term memory and sensitive to blockade with muscarinic antagonists [54]. Longer term memory was not of interest here because (i) early symptoms in AD patients pertain specifically to the short-term memory domain ([26] for review) and (ii) rodents can generally remember a previous single encounter with companions for less then 2 h [28,46,57]. Longer lasting social recognition memory either requires castration of males [6] or a training to criterion procedure [32].

Cholinergic transmission is important for cognitive performance and age-related cognitive decline is associated with the degeneration of basal forebrain cholinergic neurones (see [3,45] for review). Moreover, the formation of memory associated with social recognition appears to be modulated by acetylcholine release [64]. In experimental approaches using the conventional resident/intruder method [59], cholinomimetics or muscarinic antagonists were systemically administered after the first exposure to the juvenile rodent. Consistently, cholinergic activation via acreoline, physostigmine, nicotine [46] and tacrine [28,65] retroactively enhanced social recognition when administered systemically shortly after training. Moreover, acetylcholinesterase inhibitors like physostigmine, tacrine and galantamine increased social recognition when administered pre-training [64]. However, galantamine also increased aggressive behaviours suggesting that

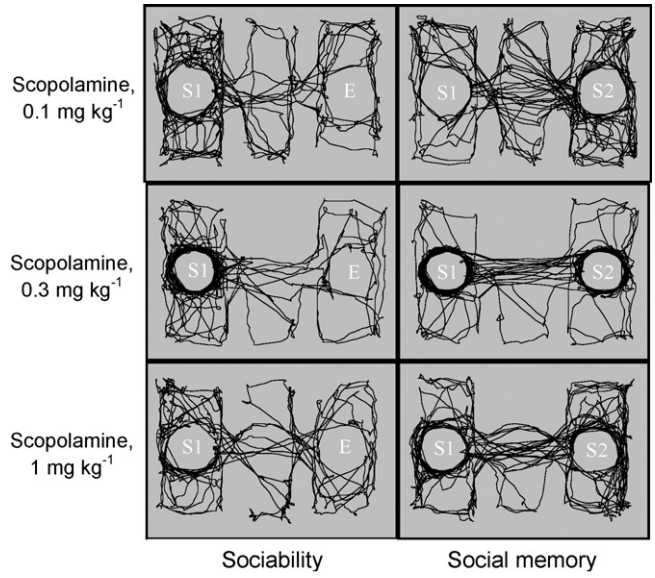

Fig. 5. Representative traces of female mice during sociability (left) and social recognition memory testing (right) when treated with different doses of scopolamine. Despite a bias for the compartment and target zone containing stranger 1 (S1 in left part as opposed to the empty (E) confinement in the right part of the chamber) during sociability, there was only social memory in the scopolamine 0.1 mg/kg group, but not when higher doses were administered (confinements for stranger 1 (S1) and stranger 2 (S2) are indicated). See Fig. 6 for group summaries.

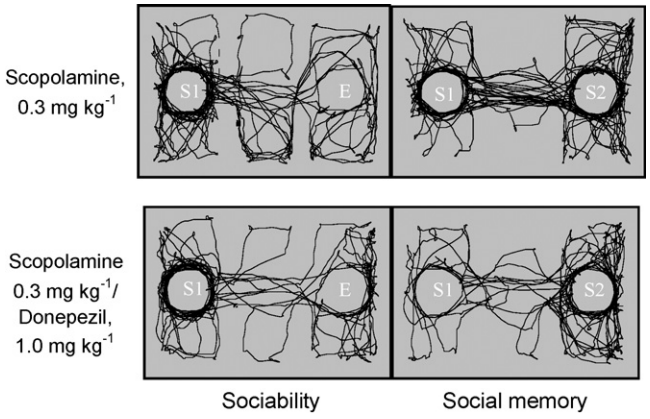

Fig. 7. Representative traces of female mice during sociability (left) and social recognition memory testing (right) when treated with scopolamine (top) or in conjunction with donepezil (bottom). Despite a clear bias for the compartment and target zone containing stranger 1 (S1 in left part as opposed to the empty (E) confinement in the right part of the chamber) during sociability, there was no social memory in the scopolamine group. This deficit was reversed by the choline esterase inhibitor donepezil. See Fig. 8 for group summaries.

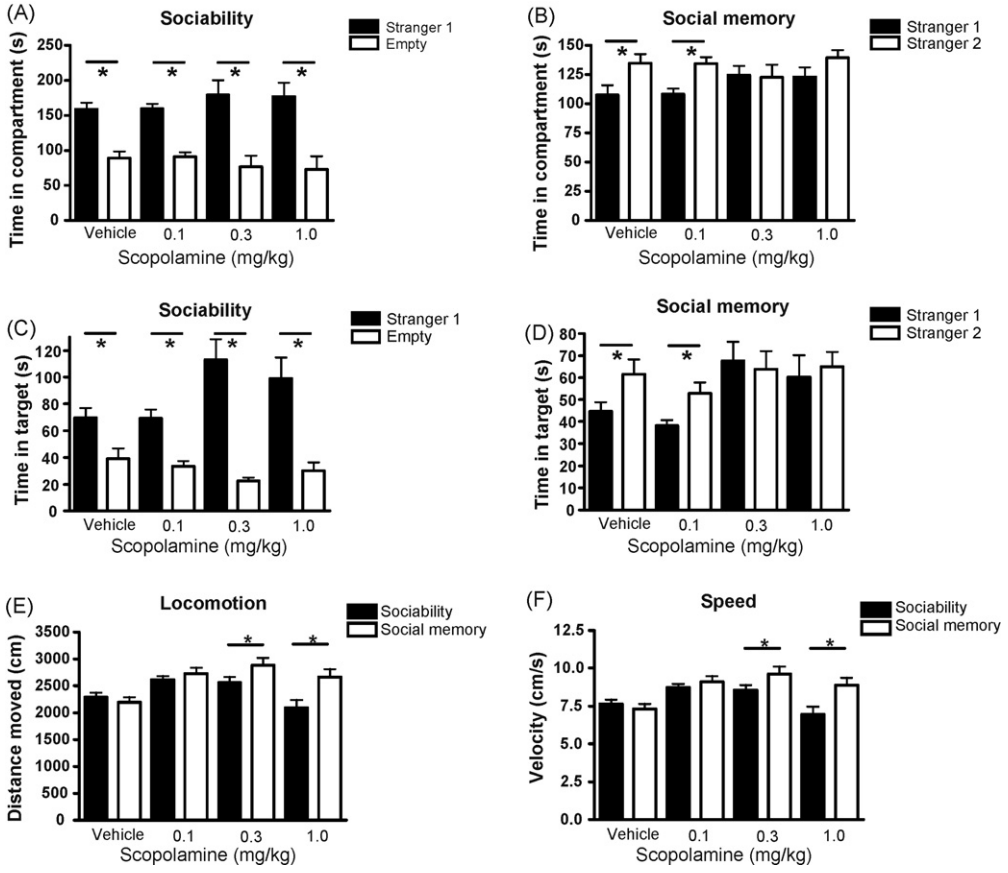

Fig. 6. Scopolamine impaired social memory, but not sociability in mice. Scopolamine was administered i.p, in various doses (0.1, 0.3, and 1.0 mg/kg) 30 min prior to the sociability test. During sociability testing, time in compartment containing stranger 1 or empty confinements (A) and respective target areas (C) were recorded and confirmed a strong bias for interaction with the companion in all groups. Social memory with a clear bias for stranger 2 over stranger 1 was observed only for vehicle and 0.1 mg/kg scopolamine in both parameters, time in compartment (B) and time in respective target zones (D). Higher doses of scopolamine (0.3 and 1 mg/kg) impaired social memory. Ambulatory locomotion measured as total distance moved (E) was increased in scopolamine-treated groups independent of dose as was running speed (E). Scopolamine-treated mice ran faster and more in the memory test than in sociability. Means + SEM for n's = 13–17. Asterisks denote significance $p < 0.05$, paired t-test, two-tailed.

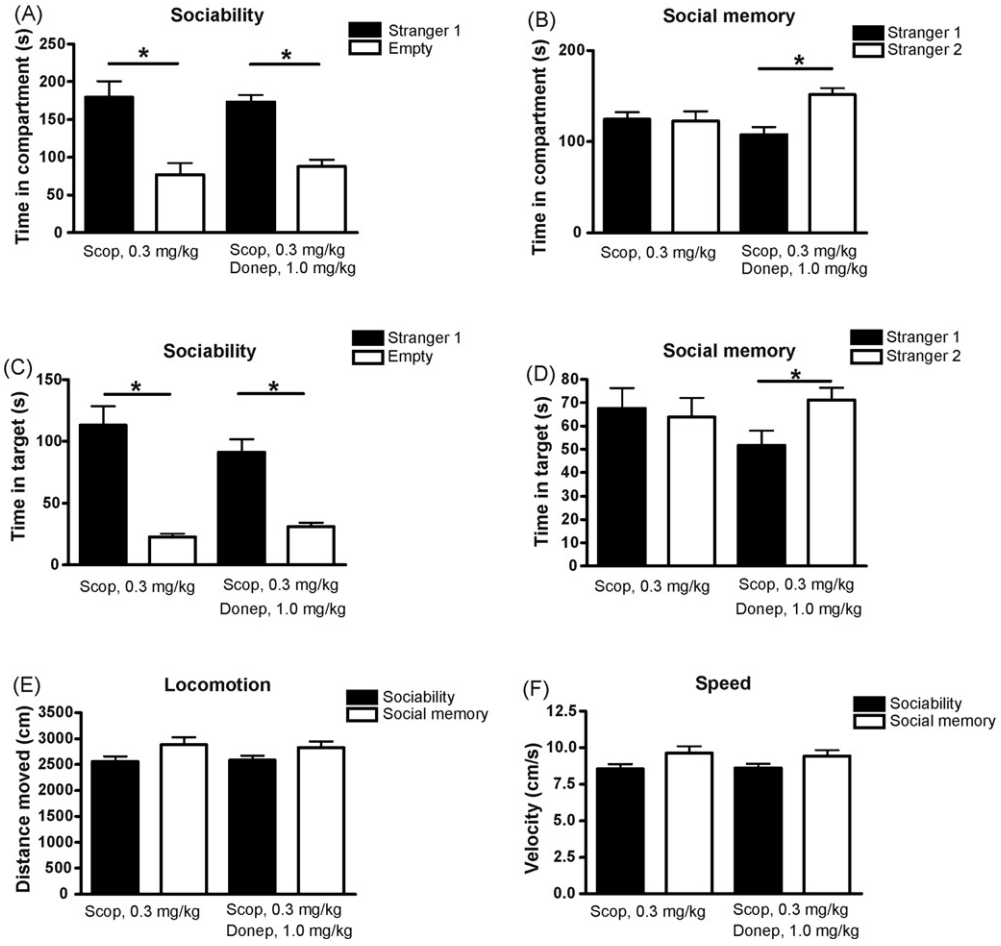

Fig. 8. Effects of denepezil on social memory deficits in scopolamine-treated mice. Mice were administered donepezil 1.0 mg/kg subcutaneously followed 1 min later by i.p. scopolamine 0.3 mg/kg. During sociability testing, time in compartment containing stranger 1 (A) and in the respective target zone (C) were recorded and confirmed a bias for the companion in all test groups. During social recognition memory testing after an interval of 30 s, scopolamine induced the previously described memory deficit in time spent in both the compartment containing stranger 2 (B) and the respective target zone (D). This deficit was successfully reversed by donepezil co-treatment. Ambulatory locomotor activity (E) running speed (E) were not different between treatment groups, but were somewhat higher during memory test. Means + SEM for n's = 13–14. Asterisks denote significance $p < 0.05$; paired t-test, two-tailed.

social behaviours may be compromised at the expense of drug-related side effects, and this might also explain the normalisation of social recognition deficits observed in hemizygous mice deficient in the vesicular acetylcholine transporter [49]. Finally, long-term administration of pilocarpine for 21 days reversed the social recognition deficit in 24-month-old rats [50] suggesting that the test is a useful model in terms of age-related cognitive decay lending further support to the notion that social interaction and recognition memory provides a useful experimental model of non-procedural non-spatial olfactory memory formation in dementia [44]. Like social transmission of food preference, social recognition memory relies on the functional activation of hippocampal-prelimbic cortical circuitries [2,32] and these circuits show early defects in AD ([37] for review).

By contrast, muscarinic receptor blockade generally impaired social recognition memory [46,64]. It therefore appears that the role of the cholinergic system in social recognition is well established. However, in ethopharmacological terms the social juvenile drug-free rodent interacts with drug-treated investigator and in turns feeds back to him/her. Any abnormality in behaviour in the drug-treated animal may therefore modify the reactions of the non-treated partner with unpredictable consequences on the behavioural interactions. We therefore felt that the recently described methodical improvements by Moy et al. [42], which draw on the ability of rodents to discriminate between a stranger and a familiar conspecific [9] would provide an advantage in allowing recording of the behaviour of the drug-treated mouse uncontaminated of any subsequent abnormality in social behaviour in the drug-free partner. Towards this end, we repeated experiments using scopolamine and found a dose-dependent impairment in short-term social recognition memory. This is unlikely due to an olfactory deficit since exploratory activity during the initial presentation of stranger 1 (sociability) was not altered. Interestingly, recognition deficits were recovered upon administration of choline esterase inhibitors [46,64] as confirmed here for donepezil. The dose administered in our experiments can also effectively reverse cognitive impairments in experimental models of AD in mice [60]. Our results contribute to the mechanism, by which this reversal is attained. Both muscarinic receptor agonists such as arecoline and RS 86 [2-ethyl-8-methyl-2,8 diazaspiro-4,5 decan-1,3 dion hydrobromide] [56] and more recently nicotinic receptor agonists acting via the α7 subtype (ABBF:[8]; A-582941: [5]; AR-R17779: [61]) have been shown to reverse cholinergic deficit in social recognition memory and could thus provide alternative cellular pathways to combat the recognition impairment.

An effective dose for scopolamine (0.3 mg/kg administered i.p.) is in the dose range for which impairments in spatial short-term memory formation have been previously reported ([54] and

citations therein). Interestingly, the deficit induced by scopolamine coincided with a drug-related increase in locomotor activity and higher speed. This is in agreement with our previous work in which similar doses also increased overall locomotion and swim speed in rats [54]. Although one might argue that this may explain the deficits, the activity increase was also present in non-amnesic doses indicating a dichotomy between the cognitive and motor effect of the drug. The latter ones appear to be more sensitive to the drug. Moreover, increased locomotor activity was observed in both sociability and social recognition memory testing, yet the former was normal at all drug doses. Finally, treatment with donepezil clearly reversed the cognitive deficit induced by scopolamine, but it did not alter locomotor activity or speed. This strongly suggests that locomotion is not a confounding element of the cognitive deficit observed here and provides compelling evidence that sensory perception remains intact in drug-treated mice. It therefore appears that scopolamine selectively affected short-term memory and not sensory perception.

The fact that locomotor activity may be independent of the amount of social activity is further supported by the observation that sociability was enhanced in diazepam treated mice which also expressed heightened activity. Several studies have provided compelling evidence that anxiolytic drugs such as diazepam increase social interaction in rats [12,13,19] and gerbils [20] with an effective dose of about 0.1 mg/kg administered i.p. This is in agreement with our dose range in mice reported here and suggests equal sensitivity to benzodiazepine receptor activation in all rodent strains. Unfortunately, short-term social recognition memory has not been investigated making it difficult to draw any firm conclusions from this data set. Since benzodiazepine receptor agonists are widely known for their memory impairing properties (see [36] for review), it may be argued that diazepam selectively blocked short-term memory in a manner similar to scopolamine [55]. Alternatively, diazepam can induce place preference [52] possibly due to its anxiolytic properties [20]. The first encounter with stranger 1 could induce a preference for this conspecific and test mice prefer this partner over stranger 2. However, this is unlikely given the fact that the time spent with the two strangers in recognition memory is equal and we did not observe a bias for any one conspecific lending some support to the former interpretation.

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