Acetylcholine elevation relieves cognitive rigidity and social deficiency in a mouse model of autism

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Supplementary Materials and Methods

Behavioral testing

Running/ jammed wheel test: mice were put in a Plexiglas cage, sized 30x30x25cm. A 14cm diameter plastic running-wheel was connected to a wall and could either turn or be jammed. At the first 4 days of the experiment, mice were put in the cage and allowed to run freely on the wheel, for 25min (days Run1 and Run2) or 15min (days Run3 and Run4, when running-habit is already acquired). Latency to start running in each day indicated acquisition of the running routine. Following acquisition, the wheel was jammed and mice behavior was monitored for two consecutive days (days Jam1 and Jam2), 15 minutes per day. Running duration was measured in the initial 10min of the last running day, and time interacting with the wheel trying to move it was measured in the initial 10min of the jammed-wheel days. Thus, adjustment to environmental change was evaluated by comparing wheel interaction time between the first jammed day and last running day. Memory of the change was evaluated by comparing the second jammed-day to the first. Hence, the ratio Jam1/Run4 was calculated for each mouse as indicator of adjustment to change, and the ratio Jam2/Jam1 was calculated for each mouse as an indicator of memory of change (Karvat and Kimchi, 2012).

'Social' running wheel test: In the last day of the wheel assay, 5 weeks old male Hsd:ICR[CD-1] stranger mouse (Harlan) was introduced to the apparatus for 10min and allowed to interact with tested BTBR mouse. Social preference was assessed by comparing time in any kind of social approach and/ or interaction with the stranger mouse, initiated by the BTBR mouse, compared to interaction time with the object (the jammed-wheel). Social preference index was calculated as (time with stranger)/(time with stranger+time with jammed wheel)×100-50 (Karvat and Kimchi, 2012).

Open-field test: Some studies reported on correlation between ACh release and locomotive activity in rodents (Day *et al*, 1991), yet others did not confirm this relationship (Thiel *et al*, 1998). In order to control for pharmacological effect on general locomotive activity and anxiety, which might influence the autism-related behaviors, the open-field test was carried on new cohort of BTBR animals that was systematically treated with saline or the drug.

Subjects were put in a cage sized 40x40x20cm for 30min. Total distance moved and time spent in the central compartment of the cage were measured using the Ethovision software.

Repetitive behaviors scoring: For repetitive grooming measure, mice were put in a clean, empty cage sized 40x40x20cm for 15min. Total cumulative duration of self-grooming episodes >2sec were scored by an observer. For repetitive digging measure, mice were put in a similar cage covered with ~1cm corn-cob-bedding (Harlan Laboratories). Cumulative durations of digging, defined as deliberately relocating bedding using the front paws, were scored by an observer.

Male-male social interaction test: Subject BTBR mice were acclimated to a clean home-cage for 20min, and then a stranger ICR male mouse (5 weeks old) was introduced into the cage, for 15min. Behaviors scored included social sniffing (snout of BTBR touched the body of ICR), chasing (snout of BTBR subject touched rear of ICR stranger and both mice moved), mounting (with and without pelvic thrust) and aggressive attacks.

Three-chamber social approach and social-novelty preference test: The test was conducted according to (Moy *et al*, 2007). The subject mouse was placed in the middle chamber of a Plexiglas box sized 70x24x29cm, divided into three chambers interconnected by retractable doors. The test was consisted of three consecutive trials of 10min. In the habituation trial, mice were allowed to explore the empty apparatus, and bias towards a chamber was monitored using Ethovision. In the social approach trial, a wire-cage (diameter: 6.5cm, height: 14cm) containing an unfamiliar 5-weeks old male ICR mouse (stranger cage) was put in one side chamber, and an empty identical wire-cage (empty cage) was placed in the opposite chamber. In the preference for social-novelty trial, an additional unfamiliar mouse (littermate of the first stranger) was placed in the wire cage that had been empty during the previous trial. Time sniffing the two cages (familiar versus novel stranger mouse) was scored using the Observer software. Social preference index was calculated as (time sniffing stranger1 cage)/(time sniffing stranger1 cage+time sniffing empty cage)×100-50. Social novelty preference index was calculated as (time sniffing stranger2 cage)/(time sniffing stranger2 cage+time sniffing stranger1 cage)×100-50. ICR mice were habituated to the wire-cages for 4 consecutive days prior to the test, 10min per day. Side-chambers containing the different stimuli were counterbalanced between subjects.

Water T-maze spatial reversal learning test: The test was based on (Dong *et al*, 2005; Guariglia *et al*, 2011), and consisted of a T-shaped Plexiglas chamber, with 3 arms sized 22x11x40cm and a center zone sized 11x11x40cm. The maze was filled with water 15cm in depth, kept on 25±1^{o}C. A platform (diameter=8cm) was submerged 0.5cm below water level, at the end of one target arm. Subjects had 10 trials during each of the 4 experiment days. The animals were put in the starting arm facing the wall, and were allowed to swim until finding the platform. If a mouse did not find the platform within 90sec, it was gently guided to it. In both cases, mice were allowed to stay on the platform for 15sec, and then rescued. Inter-trial interval was >5min. On the first and second days, the platform was located in one arm, while on the third and fourth days it was located in the opposite arm. Starting arm was identical in all days. Each trial was scored based on the first arm to which all four paws had entered; correct (target arm containing the platform) or erroneous (arm not containing the platform). In addition, latency to climb on the platform was measured using stop-watch.

References

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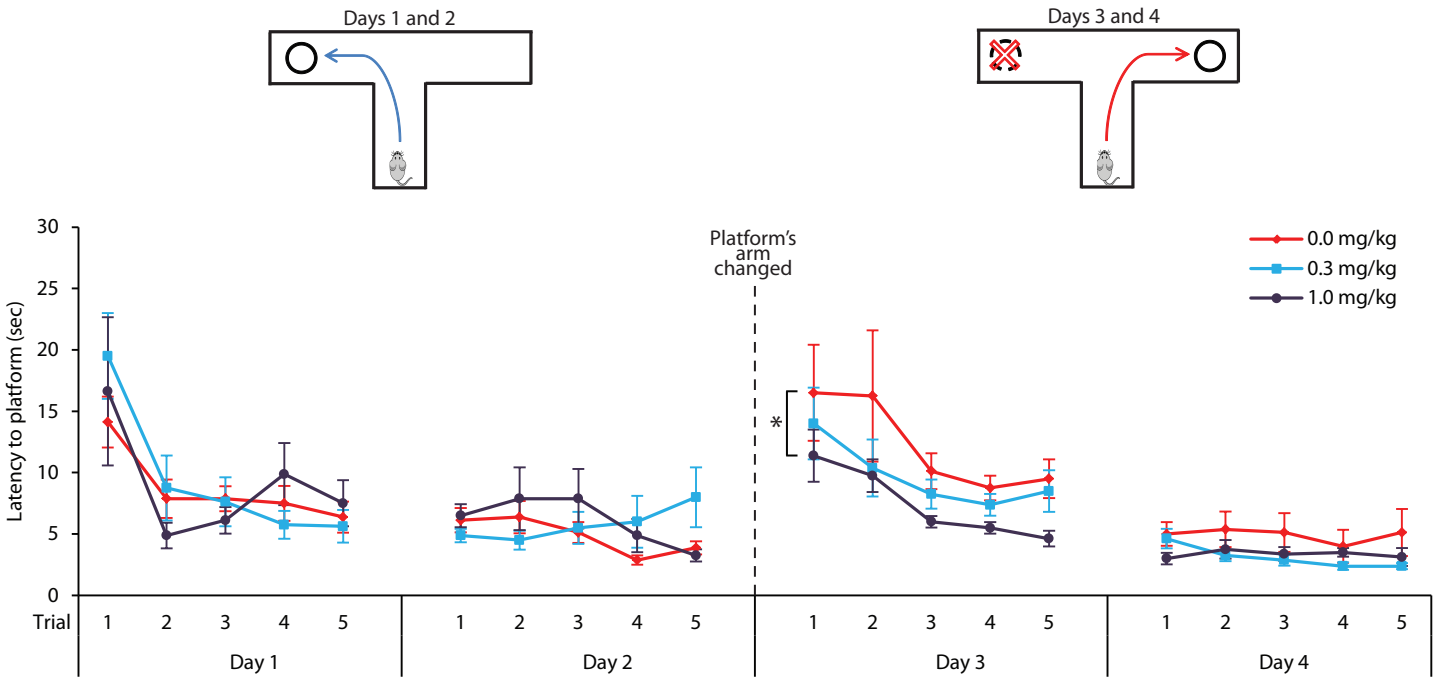

Figure S1. Intraperitoneal (i.p.) administration of Donepezil relieved cognitive rigidity in a dose dependent manner. BTBR mice were injected i.p. with Donepezil (0.3 or 1.0 mg/kg) or saline (0.0 mg/kg) for 7 days before testing and 30 min prior to each testing day in the water T-maze test. Latency to reach the escape platform in each day is presented as mean±SEM. *P<0.05. *N*=8/treatment.

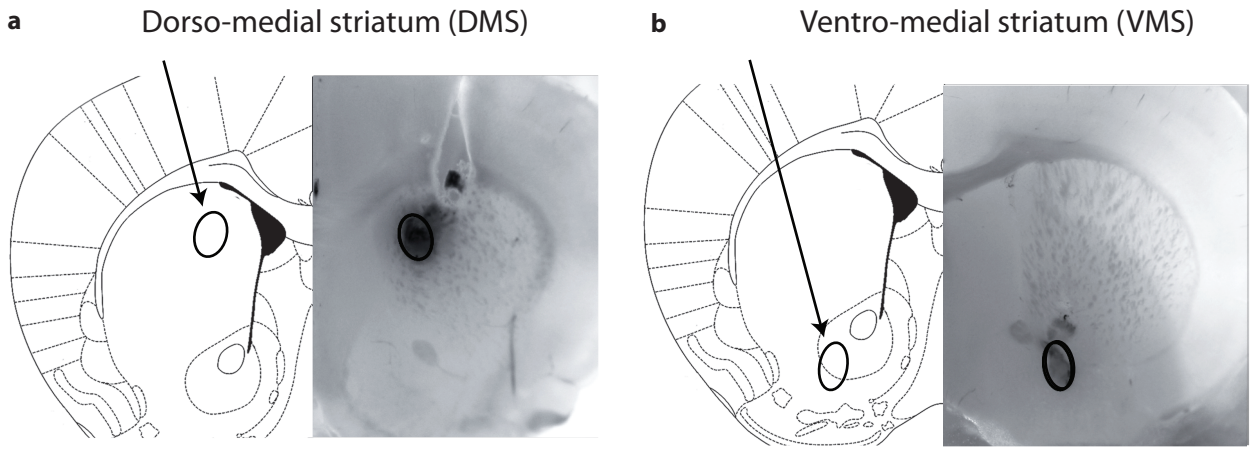

Figure S2. Location of intrastriatal infusion sites and assessment of spread.
Photomicrography (right) and schematic representation (left) of the guide cannulae tracks for infusion into the dorso-medial striatum (**a**) and into the ventro-medial striatum (**b**). The maximum spread of the drug, assessed by a dye experiment, is indicated by black circles (r=~500μm), the cannulae track and cannulae tip (injection site) are indicated by an arrow.

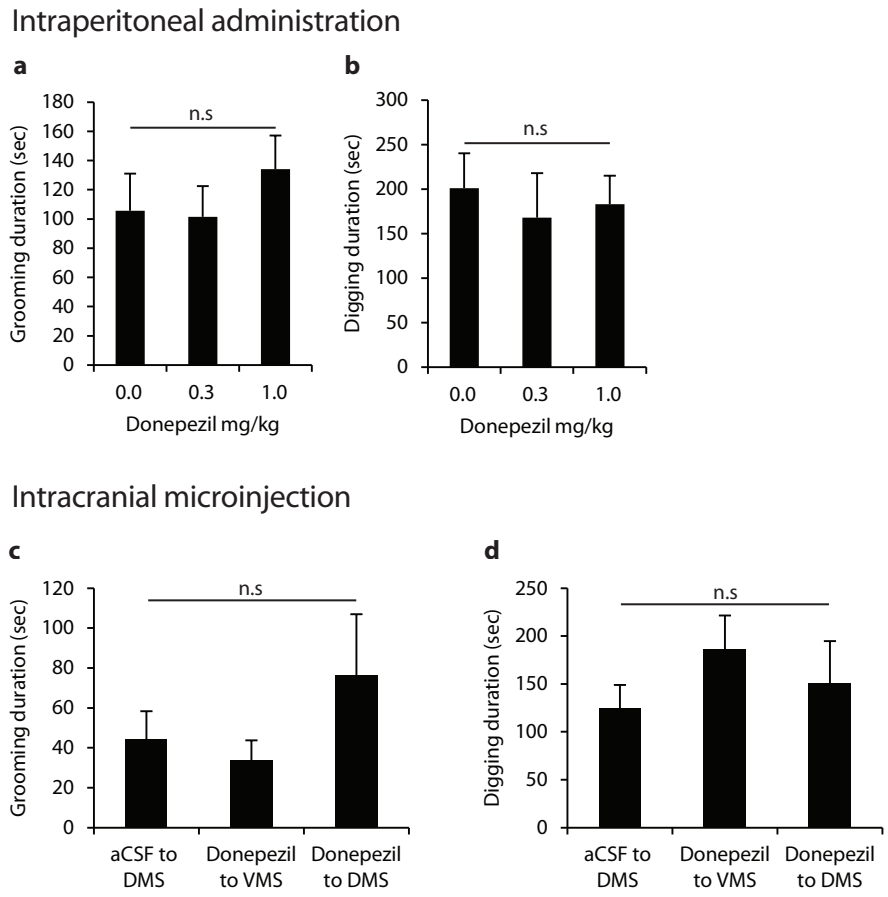

Figure S3. Administration of Donepezil did not affect stereotypical behaviors.
Grooming duration in a 15-min period in an empty cage (**a,c**) and digging duration in a 15-min period in a cage including soil-bedding (**b,d**) of mice treated with different doses of Donepezil i.p. (**a-b**) and directly into the striatum (**c-d**).
DMS= dorsomedial striatum, VMS= ventromedial striatum, aCSF = 0.5 μl artificial cerebro-spinal fluid, Donepezil= 100ng Donepezil dissolved in 0.5 μl aCSF. n.s= not significant. *N*=6-8/treatment.

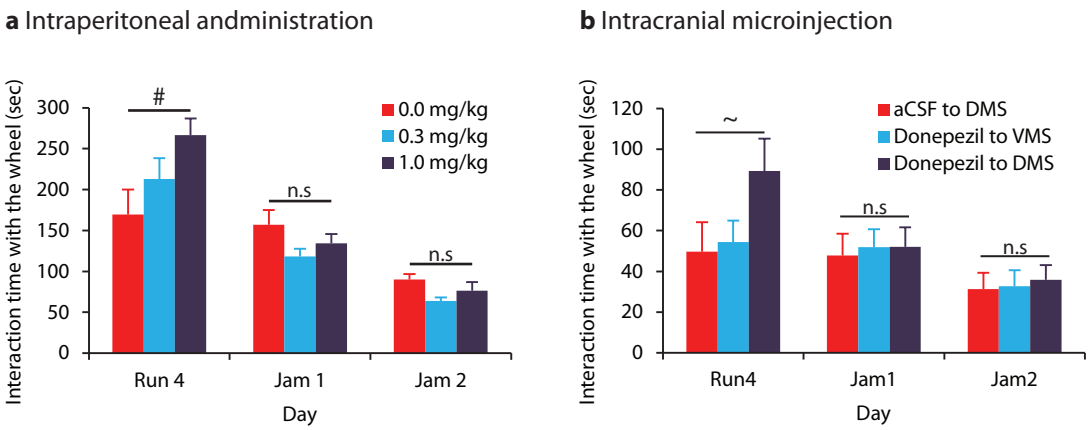

Figure S4. Effect of Denepezil on the overall running on the wheel and interaction with the jammed wheel. Cumulative interaction times with the wheel in a 10min period of the last running day (Run4) and both jammed days (Jam1 and Jam2) are presented for BTBR mice injected intraperitoneally (**a**) or intracranially into the striatum (**b**). DMS= dorsomedial striatum, VMS= ventromedial striatum, aCSF = 0.5μl artificial cerebro-spinal fluid, Donepezil= 100ng Donepezil dissolved in 0.5μl aCSF. Data is presented as mean±SEM. #P=0.06, ~P=0.08, n.s= not significant. *N*=6-8/treatment.

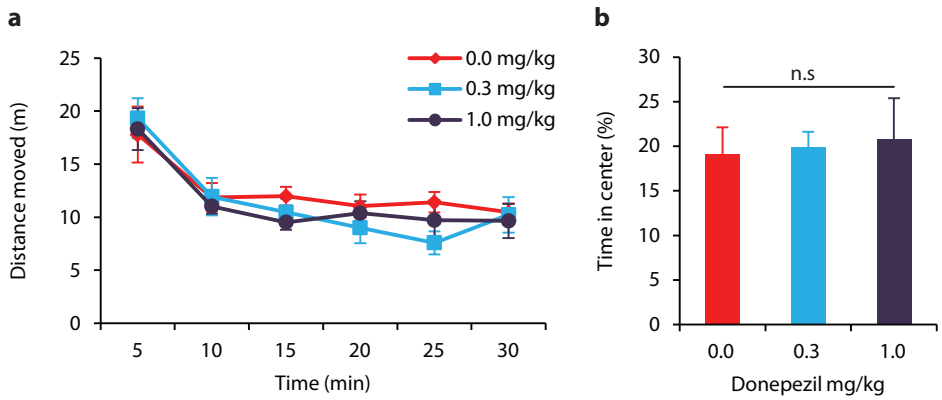

Figure S5. Intraperitoneal administration of Donepezil did not affect general activity and anxiety-related behaviors.

BTBR mice were i.p. injected with Donepezil (0.3 or 1.0 mg/kg) or saline (0.0 mg/kg) for 7 days before testing and 30 min prior to each testing day (**a**) General activity: distance moved in 30 minutes in the open-field test. (b) Precentage of time spent in the central compartment of the open-field.
Data is presented as mean±SEM. n.s= not significant. *N*=8/treatment.

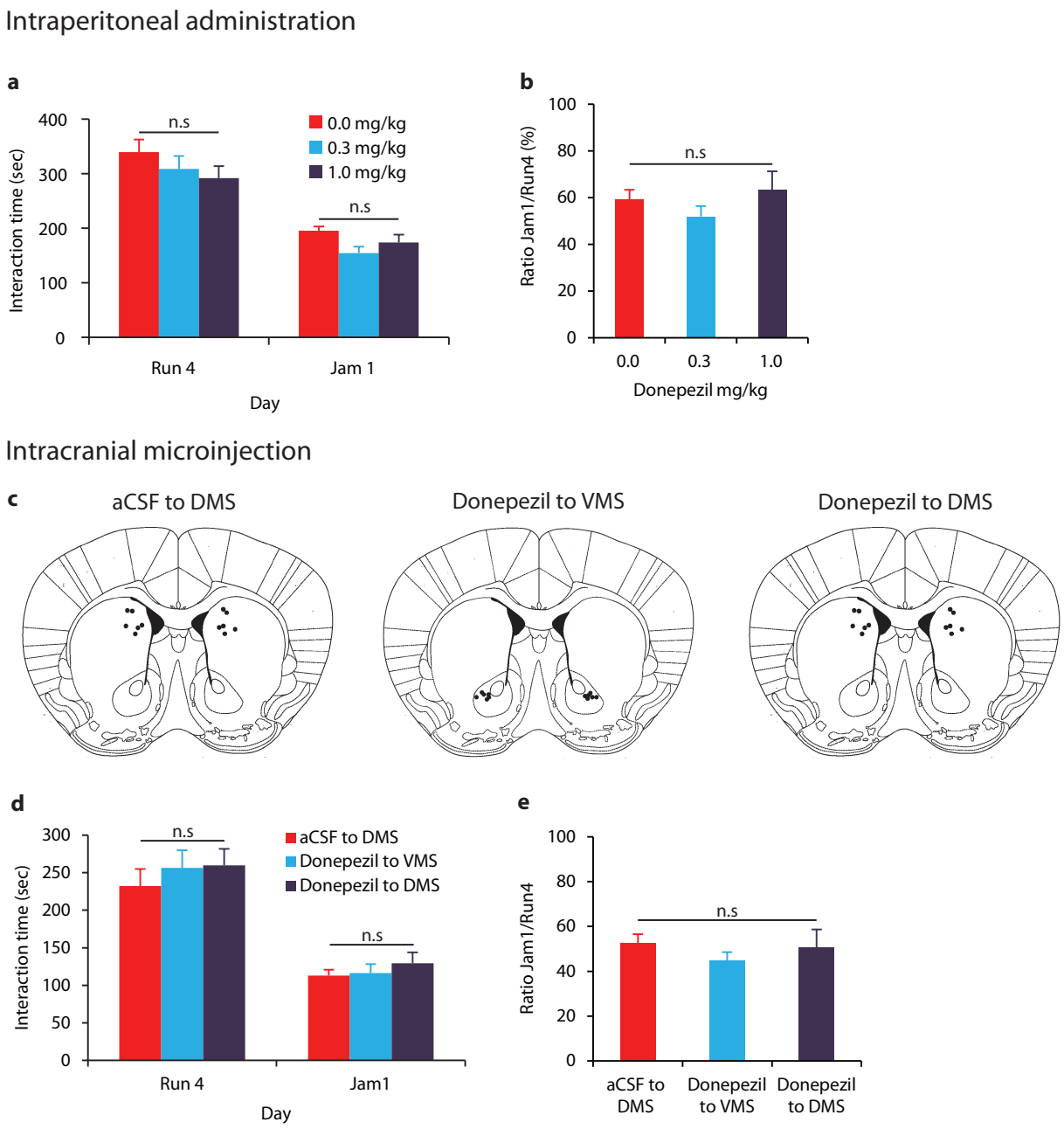

Figure S6. Treatment with Donepezil did not affect the overall running in the wheel and interaction with the jammed wheel in mice from the control strain FVB .
FVB mice were treated in similiar fashion to the treatment of BTBR mice, in both intraperitoneally (**a-b**, *N*=8/treatment) and intracranially (**c-e**, *N*=7/treatment), and tested in the jammed/ running wheel assay). (**a,d**) Cumulative duration of interaction with the wheel in the last running day (Run4) and first jammed day (Jam1). (**b,e**) The ratio between interaction time in the jammed day to the last running day, indicating adjustment to change. (**c**) Illustration of the tips of cannula implanted to FVB mice. DMS= dorsomedial striatum, VMS= ventromedial striatum, aCSF=0.5µl artifical cerebro-spinal fluid, Donepezil=100ng Donepezil dissolved in 0.5µl aCSF. n.s=not significant.