Effects of advanced paternal age on trajectories of social behavior in offspring.

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/gbb.12227

Abstract: Our study is the first investigation of the effects of advanced paternal age (APA) on the developmental trajectory of social behavior in rodent offspring. Given the strong epidemiological association between APA and sexually-dimorphic neurodevelopmental disorders that are characterized by abnormalities in social behavior (autism, schizophrenia), we assessed sociability in male and female inbred mice (C57BL/6J) across postnatal development (N = 104) in relation to paternal age. We found differences in early social behavior in both male and female offspring of older breeders, with differences in this social domain persisting into adulthood in males only. We showed that these social deficits were not present in the fathers of these offspring, confirming a *de novo* origin of an altered social trajectory in the offspring generation. Our results, highly novel in rodent research, support the epidemiological observations in humans and provide evidence for a causal link between APA, age-related changes in the paternal sperm DNA and neurodevelopmental disorders in their offspring.

Introduction:

Advanced paternal age (APA) is linked to a host of adverse outcomes in children. Psychiatric problems, especially those with a neurodevelopmental background, constitute a prominent group among these; APA has been shown to be a risk factor for autism spectrum disorders (ASD) (Reichenberg et al., 2006), schizophrenia (Malaspina, 2001), attention deficit and hyperactivity disorder (ADHD) (D'Onofrio et al., 2014) and early-onset bipolar disorder (Frans et al., 2008). This suggests that the effects of APA on the mental health are likely to be mediated by a disturbance in early brain development. The molecular mechanisms mediating the APA effects remain unknown. The predominant hypothesis in the field postulates age-related accumulation of *de novo* mutations in paternal sperm DNA (Kong et al., 2012), with some evidence suggesting that epigenetic changes in these cells could also be implicated (Perrin, Brown, & Malaspina, 2007; Smith et al., 2012). However, before molecular mechanisms can be fully investigated and interpreted, it is essential that the behavioral phenotype(s) of APA are fully characterized.

We investigated the effects of APA on social behavior in offspring using mouse models. Our study design addressed a number of problematic aspects in the mouse APA research conducted to date, including: (i) validating *de novo* origins of APA effects in the offspring generation; several lines of research suggested they are likely to be mediated by pre-existing differences in males that decide to delay fatherhood (Miller et al., 2011; Petersen, Mortensen, & Pedersen, 2011); (ii) developmental trajectory of these effects; given the neurodevelopmental nature of the disorders arising as a consequence of APA, one should expect relevant animal models to manifest differences in early development, as well as in

adulthood (Foldi, Eyles, McGrath, & Burne, 2010); (iii) potential sex differences in the effects of APA; given sexual dimorphism in rates and presentation of the neurodevelopmental disorders for which APA has been shown to be a risk factor (e.g. higher prevalence for autism and schizophrenia in males, earlier onset of schizophrenia and more severe disease course in males). Our design addressed (i) by controlling for paternal social behavior. To assess (ii) we conducted social behavior tests both early in development and in adulthood. Finally, to resolve (iii), our sample included animals of both sexes.

Materials and Methods:

Study design

We assessed behavior in the Generation 1 offspring of "young" (8 weeks old at breeding; n=8; YF), "old" (40 weeks old at breeding; n=8; OF) and "very old" fathers (48 weeks old at breeding; n=8; VOF). These paternal ages approximate to young adult (20's) through to middle age (mid-40's) men ("Life span as a biomarker," 2011). The age of all dams was 8 weeks at breeding, eliminating confounding effects of the maternal age. Early developmental (males and females combined; n=15-16/group) and adulthood (n=10/sex/group) testing was conducting on separate sets of Generation 1 animals (which were, however, littermates). This approach necessitated group, rather than individual comparisons, however, gave us confidence that the patterns observed later in life do not represent carry-over effects of early handling. Additionally, to control for the possible inherited behavioral effects, we assessed sociability in adult Generation 0 male breeders. For a schematic of the study design see Fig. 1. To investigate if any possible APA effects affect specifically offspring sociability, or have a broader behavioral effect, apart from social interaction we also assessed baseline locomotor activity (homecage), exploration (holeboard), anxiety (open field), motor co-ordination and

learning (rotarod) and olfaction (olfactory habituation / dishabituation). See Supplementary Materials (Table S1) for all protocols.

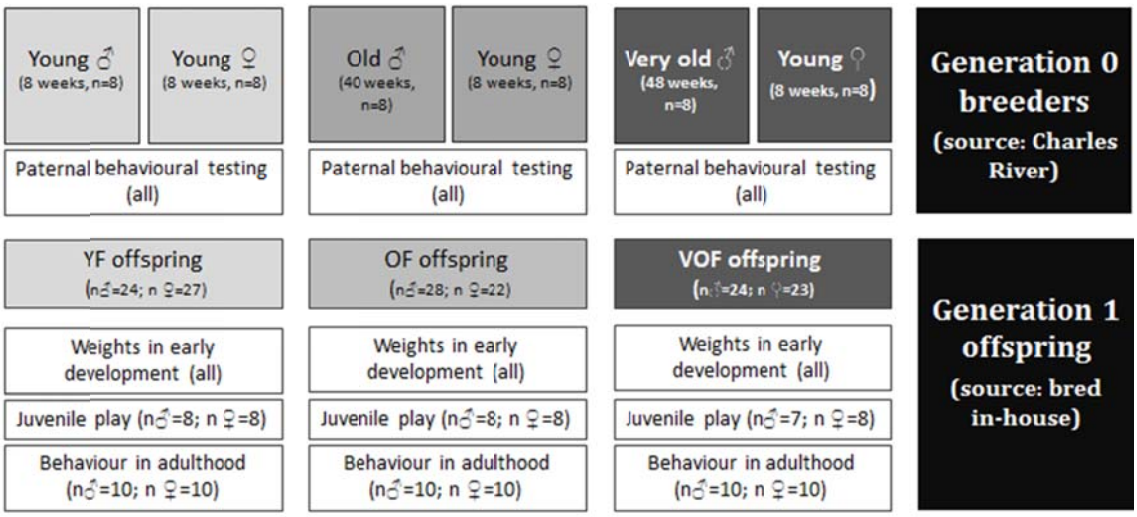

Figure 1. Study design. In Generation 1, all animals were weighed at postnatal days 6, 10, 15, 20 and 29. Separate subsets of these mice were subsequently used for behavioral testing at PND31 (juvenile play assessment) and in adulthood (comprehensive behavioral screen).

Animals

Male and female C57BL/6J mice (Generation 0: males aged 6 (n=8), 38 (n=8) and 46 weeks (n=8) and females aged 6 weeks (n=24)) were purchased from Charles River (Margate, UK) and bred in-house after a 2-week habituation period. We ensured all males were sexually-experienced by the time of mating, by providing them with a 3-day long exposure to a female conspecific (females used at this stage were not re-used for any other purposes in our study). All males were paired with a single virgin female and removed from the cage after 2 weeks (i.e. about a week before the pups were born) to minimize/prevent any paternal behavioral influence on the pups and/or the mother-offspring interaction. The size and sex ratio of all the Generation 1 litters was recorded at postnatal day 6. All offspring were weighed at postnatal days 6, 10, 15, 20 and 29 and at culling, weaned at postnatal day 28 (2-4 mice/cage; males and females housed separately) and subsequently tested (for details see table S1 and sections below). Conspecifics for social tests (juvenile play, social investigation) were bought in a

week before testing to allow for a habituation period; these animals were group-housed (2-4 mice/cage; males and females housed separately), and at all times kept in a different holding room, to prevent any exposure by the test animals before social testing). All mice were housed in Tecniplast cages (32cm x 16cm x 14cm) with sawdust (Litaspen premium, Datesand Ltd, Manchester, UK) and, unless indicated otherwise, cage enrichment was provided consisting of Sizzlenest (Datesand Ltd, Manchester, UK) and a cardboard shelter (LBS Biotech, Horley, UK).Test mice were individually housed prior to testing (24h for juvenile, a week for adult mice). Previous research conducted in our laboratory indicated that singly housing has minimal effects on the behavior of C57BL/6J mice and avoids confounds associated with group housing, such as the establishment of social hierarchies which could influence the controlled assessment of social behaviors (Lad et al., 2010). All mice had *ad libitum* access to water and food (Rat and Mouse No. 3 Diet for breeders and No. 1 for all other mice; Special Diet Services, Essex, UK). All housing and experimental procedures were performed in compliance with the local ethical review panel of King's College London, and the U.K. Home Office Animals Scientific Procedures Act 1986. The work was carried out under license (PPL: 70/7184) and all efforts were made to minimize animal suffering and to reduce the number of animals used.

Juvenile social play

A total of 44 animals from Generation 1 were used for juvenile play testing (23M + 21 F; n=8 in all paternal age/sex groups, apart from VOF males with n=7). These animals were not used in any subsequent behavioral testing, in order to avoid potentially confounding effects of early testing on development. All testing took place during the light phase. The mice were removed from their litter cages and singly housed on PND31, in a fresh cage with sawdust but no other enrichment, and with a free access to food and water. After 24h they were

moved (1 animal at a time) to the dark test room lit with red light from 4 red cluster lights (LED cluster red light No. 310-6757; RS Components Northants, UK) of approximate wavelength 705 nm which provided minimal red light to allow video recording of the test. Test mice (in their homecage) were habituated to the test room for 5 min and then a novel, age-matched, same-sex conspecific mouse was added to the test cage and their interactions were recorded for 10 min. Social play behavior was scored from these recordings by an experimenter blind to the paternal age groups (for details of the measures scored see Table S2 (Terranova & Laviola, 2005)). The inter-rater reliability, determined using inter-class correlation, had a mean of 0.902 across all social behavior measures. At the end of the test, the conspecific was returned to its homecage, and enrichment was added to the test animal's cage.

Social interaction in adulthood

Generation 0 male breeders

The Gen0 male breeders (n=8/group) were tested after pairing-up period to ensure no knock-on effects of their behavioral testing on females and their offspring. The animals were singly-housed at least one week prior to testing and only handled whilst cage-cleaning (cages were never cleaned the day before, or on the testing day to minimize the potential effects of cage disturbance on the behavior of the mice). All testing took place during the light phase, in a dimly-lit room. The test was performed and coded as described in the original protocol (Winslow, 2003), with the only differences that (i) the camera was placed overhead, rather than at 15-20° angle, and (ii) second (recognition) trial was omitted. Conspecifics (age-matched and counterbalances between the experimental groups) were introduced to the test animal's homecage for 5 min, and their interactions were recorded. Social interaction was

scored from these recordings by an experimenter blind to the paternal age groups (for details of the measures scored see Table S3 and Winslow, 2003).

Generation 1 offspring

All of the procedures were carried out as described above and in the Table S1. 60 animals from Generation 1 aged 12 weeks were used for adult behavioral testing (30M + 30F; n=10/sex/paternal age group). The animals were removed from their litter cages and singly housed at 8 weeks of age.

Data processing and analysis

All statistical analyses were run using R (version 2.15.2).

Linear models were applied to all of the tests. When non-normal data distribution was observed other models (Cox proportional hazard for censored, or mixture model for binomial data) were tested; however, given that statistical power was not affected by switching to these models, linear models were used in all analyses as most parsimonious. For all of these measures, effects of both paternal age (treated as a numeric variable) and sex were considered in the model. All significant tests were followed up with post-hoc analyses (Tukey honest significant difference). Levene's test was used to investigate homogeneity of variances in different paternal age groups. Cohen's f^2 was chosen to report effect sizes in our data.

Given that in some instances more than one animal / litter was used in behavioral testing, we run hierarchical models to ensure that any clustering we observed in our data could be accounted for. Paternal ID was entered as a random effect, and paternal age and sex (juvenile

only; in adults analysis was carried out separately for males and females) as fixed effects. These analyses were run for all social data.

Durations and frequencies of individual behaviors in the juvenile play and adult social investigation tests were summed up within behavioral categories, as described previously (Terranova & Laviola, 2005; Winslow, 2003). In addition, mean durations of a single bout of an interaction were computed by dividing the total interaction time by frequency of these behaviors in a given animal. Analyses were performed on both individual behavior categories (investigative, affiliative, play/interaction-soliciting), and their sum (social behaviors).

Different animals (yet born to the same set of breeders) were used in juvenile and adulthood behavioral assessments. This approach was chosen due to otherwise potential confounding knock-on effects of early handling and single-housing with no enrichment provided, inevitable in the juvenile sociability assessment. The animals from within each litter were assigned to either juvenile or adulthood testing batch at random. To validate that this approach could approximate a longitudinal design (avoiding the confounding effects described above), we verified if early gross development – as measured by body weight – clustered within litters, and if these effects went over and above those caused by paternal age. We run hierarchical model, with paternal ID entered as a random effect, and paternal age and sex as fixed effects.

Results:

There were no significant differences in the social behavior of the YF, OF and VOF Generation 0 male breeders (Table 1). We observed no differences in any other behavioral domain in these animals (data not shown), apart from exploration (Table S4). Paternal age

had no effect on the size (p = 0.375) or the sex ratio (p = 0.456) of the litters (Table S5). Offspring in Generation 1 showed no differences in any behavioral domain (locomotor activity, olfaction, anxiety; data not shown) apart from social interaction (see below) and motor learning. Although male offspring of very old breeders showed markedly worse performance at the beginning of the rotarod task, these differences disappeared by the second day testing, suggesting a mild deficit in motor co-ordination, but no profound motor impairment (Table S6).

		Frequency (count)	Duration (s) (total)	Duration (s) (single bout)
Total social behaviors	**YF**	M=52.75 (*7.60*)	M=69.91 (*8.75*)	M=1.50 (*0.08*)
	OF	M=42.75 (*3.38*)	M=61.72 (*6.70*)	M=1.58 (*0.11*)
	VOF	M=41.00 (*4.20*)	M=54.53 (*8.14*)	M=1.47 (*0.14*)
	p-value	F(1,22)=2.454, p=0.132	F(1,22)=1.979, p=0.173	F(1,22)=0.036, p=0.852
Investigative Behaviors	**YF**	M=42.63 (*5.56*)	M=60.94 (*6.20*)	M=1.48 (*0.08*)
	OF	M=36.13 (*2.84*)	M=58.60 (*6.46*)	M=1.63 (*0.13*)
	VOF	M=33.75 (*3.47*)	M=49.65 (*7.64*)	M=1.45 (*0.14*)
	p-value	F(1,22)=2.406, p=0.135	F(1,22)=1.44, p=0.243	F(1,22)=0.029, p=0.866
Affiliative Behaviors	**YF**	M=5.25 (*2.03*)	M=8.97 (*3.55*)	M=2.38 (*0.73*)
	OF	M=3.38 (*1.08*)	M=3.12 (*0.95*)	M=1.08 (*0.27*)
	VOF	M=2.75 (*1.32*)	M=4.89 (*2.82*)	M=1.62 (*0.50*)
	p-value	F(1,22)=1.386, p=0.252	F(1,22)=1.145, p=0.296	F(1,16)=1.053, p=0.320
Interaction-soliciting behaviors	**YF**	M=4.88 (*0.97*)	-	-
	OF	M=3.25 (*1.05*)	-	-
	VOF	M=4.50 (*0.87*)	-	-
	p-value	F(1,22)=0.074, p=0.788	-	-
Inactive	**YF**	M=0	M=0	M=0
	OF	M=0	M=0	M=0
	VOF	M=0	M=0	M=0
	p-value	-	-	-

Table 1. Descriptive (mean and standard error in brackets) and p-value statistics for social investigation parameters measured in male breeders, total social behaviors and grouped by interaction type. For interaction-soliciting items only frequency measures could be reliably determined; 'inactive' was the only item in this domain where durations could be computed, and statistics are presented in the table. ANOVA test was used for all comparisons (df(1,22)).

Hierarchical models confirmed that paternal ID (equivalent with litter, as each breeder was used to impregnate one female only) introduced a considerable degree of clustering in early

developmental weights (Table S7), but not in either juvenile or adulthood social behavior (Table S8). Paternal age had no effect on weights either in early development or at culling, and sex effects on weights emerged at postnatal day 20 (data not shown).

Due to no significant sex differences in juvenile social play (Table 2), all juvenile analyses were performed on pooled male and female data. APA altered social behavior early in development, with OF and VOF offspring showing significantly higher frequencies ($p=0.007$; $f=0.206$) and durations ($p=0.016$; $f=0.162$) of social behaviors at postnatal day 32. Furthermore, the mean duration of a single interactive social bout was longer in the offspring of older fathers ($p=0.005$; $f=0.212$). Individual behavior types (affiliative, play-soliciting and investigative) showed the same profiles in response to APA (Fig. 2), with only the duration of interactive bouts not significantly different between paternal age groups. These effects were linear across the groups (apart from play-soliciting), with the biggest difference observed between YF and VOF mice (Table 2).

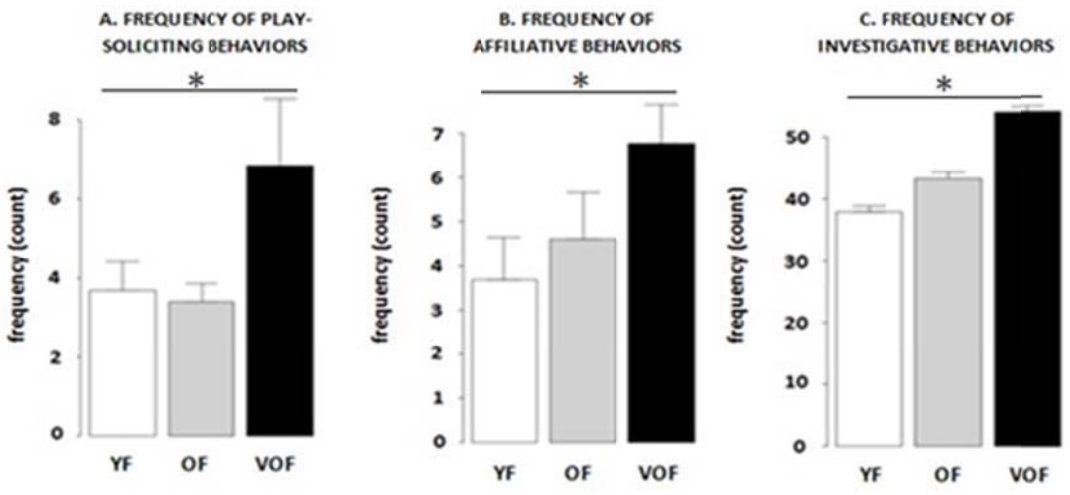

Figure 2. Frequency of play-soliciting (A), affiliative (B) and investigative (C) behaviors in juvenile offspring (mean + SEM). Paternal age had a significant effect on all of these measures ($p_{play}=0.045$, $p_{aff}=0.034$, $p_{inv}=0.025$), with lower values observed in YF (white), as compared to OF (grey) and VOF (black) offspring; there were no sex effects observed.

		Frequency (count)	Duration (s)	Duration (s)

			(total)	(single bout)
Total social behaviors	**YF**	M=45.250 *(4.533)* Mdn=40.5 *(22.980)*	M=102.970 *(14.007)* Mdn=79.615 *(45.390)*	M=2.177 *(0.101)* Mdn=2.166 *(0.312)*
	OF	M=51.267 *(5.159)* Mdn=52.0 *(22.239)*	M=157.241 *(28.136)* Mdn=150.920 *(56.947)*	M=2.430 *(0.113)* Mdn=2.541 *(0.255)*
	VOF	M=67.615 *(7.014)* Mdn=63.0 *(35.582)*	M=178.178 *(20.171)* Mdn=189.220 *(82.047)*	M=2.616 *(0.110)* Mdn=2.443 *(0.322)*
	p-values	F_{PA} (1,41)=7.789, p_{PA}= 0.008** F_{sex} (1,41)=0.267, p_{sex} = 0.608 p_{YF-OF}= 0.708; p_{YF-VOF} = 0.018 p_{OF-VOF} = 0.114	F_{PA} (1,41)=6.56, p_{PA} = 0.014* F_{sex} (1,41)=2.65, p_{sex} = 0.111 p_{YF-OF}= 0.170 p_{YF-VOF} = 0.048 p_{OF-VOF} = 0.781	F_{PA} (1,40)=8.593, p_{PA} = 0.006** F_{sex} (1,40)=0.929, p_{sex} = 0.341 p_{YF-OF}= 0.381 p_{YF-VOF} = 0.980 p_{OF-VOF} = 0.530
	Levene's test	Df(2,41); F=0.917; p=0.408	Df(2,41); F=0.243; p=0.785	Df(2,41); F=0.955; p=0.393
Investigative Behaviors	**YF**	M=37.875 *(3.708)* Mdn=34 *(15.567)*	M=91.133 *(12.883)* Mdn=75.615 *(43.789)*	M=0.455 *(0.0241)* Mdn=0.451 *(0.0730)*
	OF	M=43.267 *(4.864)* Mdn=45 *(16.309)*	M=141.537 *(29.034)* Mdn=136.480 *(59.423)*	M=0.407 *(0.0466)* Mdn=0.378 *(0.0677)*
	VOF	M=54 *(6.121)* Mdn=52 *(31.135)*	M=153.809 *(18.784)* Mdn=166.290 *(70.468)*	M=0.361 *(0.0164)* Mdn=0.357 *(0.0809)*
	p-value	F_{PA} (1,42)=5.413, p_{PA} =0.0249* p_{YF-OF}= 0.700 p_{YF-VOF} = 0.063 p_{OF-VOF} = 0.289	F_{PA} (1,42)=4.5, p_{PA} = 0.0398* p_{YF-OF}= 0.209 p_{YF-VOF} = 0.111 p_{OF-VOF} = 0.917	F_{PA} (1,42)=0.091, p_{PA} = 0.764
	Levene's test	Df(2,41); F=1.961; p=0.154	Df(2,41); F=0.335; p=0.717	Df(2,41); F=0.976; p=0.385
Affiliative Behaviors	**YF**	M=3.688 *(0.952)* Mdn=2.5 *(3.706)*	M=6.369 *(1.540)* Mdn=4.005 *(5.938)*	M=0.575 *(0.0532)* Mdn=0.599 *(0.228)*
	OF	M=4.600 *(1.059)* Mdn=3 *(2.965)*	M=10.339 *(2.479)* Mdn=6.780 *(8.480)*	M=0.532 *(0.0576)* Mdn=0.488 *(0.0632)*
	VOF	M=6.769 *(0.878)* Mdn=6 *(2.965)*	M=13.733 *(2.134)* Mdn=10.730 *(8.110)*	M=0.537 *(0.0368)* Mdn=0.480 *(0.0563)*
	p-value	F_{PA} (1,42)=4.831, p_{PA} = 0.0335* p_{YF-OF}= 0.777 p_{YF-VOF} = 0.082 p_{OF-VOF} = 0.287	F_{PA} (1,42)=6.412, p_{PA} = 0.0152* p_{YF-OF}= 0.353 p_{YF-VOF} = 0.0436 p_{OF-VOF} = 0.501	F_{PA} (1,37)=0.989, p_{PA} = 0.989
	Levene's test	Df(2,41); F=0.334; p=0.718	Df(2,41); F=0.776; p=0.467	Df(2,36); F=1.460; p=0.246
Play-soliciting behaviors	**YF**	M=3.688 *(0.734)* Mdn=3 *(2.965)*	M=5.468 *(1.301)* Mdn=3.93 *(4.967)*	M=0.882 *(0.102)* Mdn=0.775 *(0.297)*
	OF	M=3.400 *(0.466)* Mdn=3 *(1.483)*	M=5.365 *(0.876)* Mdn=4.76 *(3.707)*	M=0.688 *(0.0509)* Mdn=0.671 *(0.210)*
	VOF	M=6.846 *(1.683)* Mdn=6 *(4.448)*	M=10.634 *(2.555)* Mdn=9.09 *(5.990)*	M=0.661 *(0.0269)* Mdn=0.674 *(0.123)*
	p-value	F_{PA} (1,42)=4.253, p_{PA} =0.0454* p_{YF-OF}= 0.977 p_{YF-VOF} = 0.086 p_{OF-VOF} = 0.061	F_{PA} (1,42)=4.518, p_{PA} = 0.0395* p_{YF-OF}= 0.999 p_{YF-VOF} = 0.079 p_{OF-VOF} = 0.077	F_{PA} (1,41)=2.365,, p_{PA} = 0.132

	Levene's test	Df(2,41); F=2.343; p=0.109	Df(2,41); F=1.930; p=0.158	Df(2,40); F=2.424; p=0.102

Table 2. Descriptive (mean and standard error in brackets), ANOVAs' and Levene's test statistics for juvenile play parameters, total social behaviors and grouped by interaction type. ANOVA test was used for all comparisons. For all significant results post-hoc analyses were run (Tukey HSD test), and pairwise group comparisons reported. Means (M) are presented with standard errors, medians (Mdn) with median absolute deviation (n=16/group/sex groups, apart from VOF, where n=15). Levene's test was run to determine if there were any significant differences between the groups' variances.

We observed decreased social behavior in the adult (10 weeks) male OF/VOF offspring. The effect of APA on adult social behavior was mainly evident in the investigative behaviors (Fig. 3). APA had a significant, or borderline-significant effect on all the parameters in this domain, including single bout duration (p=0.039; f^2=0.175), total duration (p=0.007; f^2=0.305) and frequency (p=0.076; f^2=0.122). These effects were linear across the groups, with the biggest difference observed between YF and VOF mice. Affiliative and interaction-soliciting behaviors were not significantly associated with paternal age. There were no effects of APA on social behavior in adult females, nor on the duration of interactive bout in either of the sexes (Table 3).

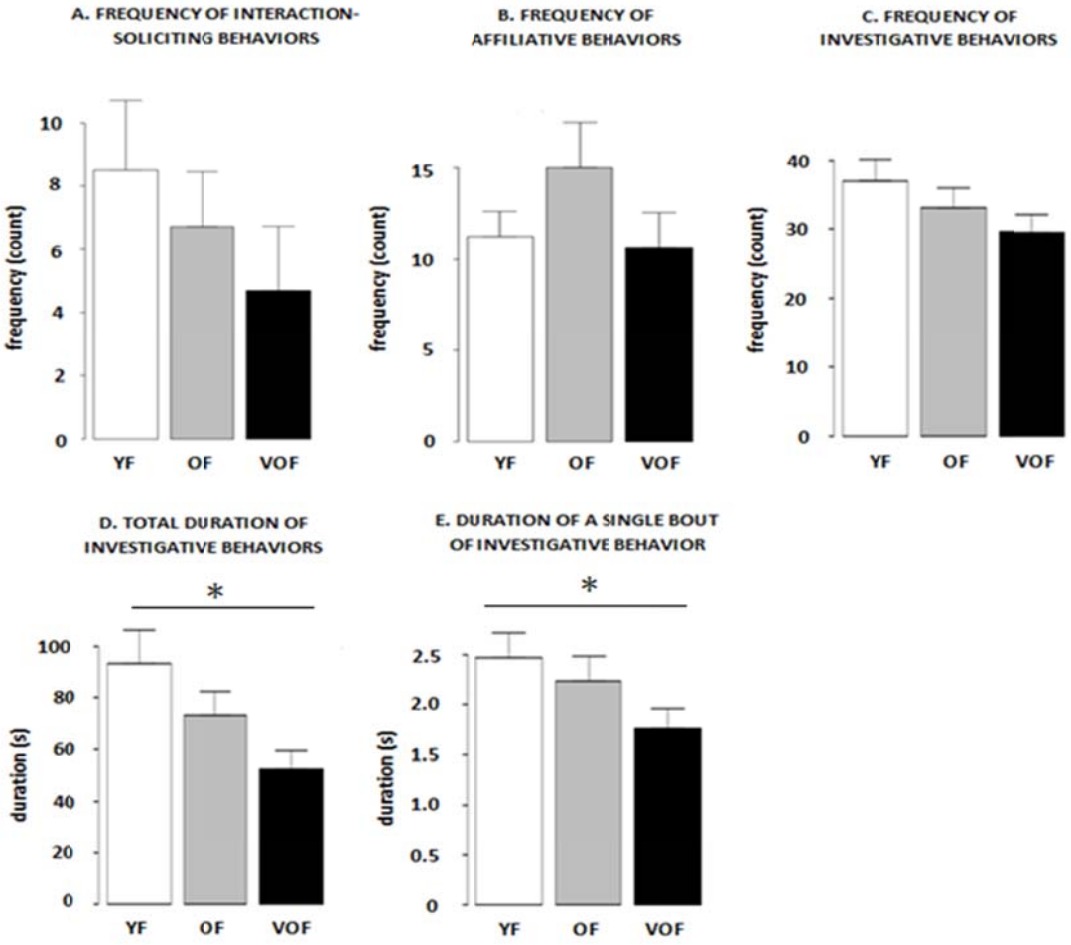

Figure 3. Frequency of interaction-soliciting (A), affiliative (B) and investigative (C) behaviors (top panel) and total duration (D) and duration of a single bout of investigative behaviors (E) (bottom panel) in Gen1 male adult (>10 weeks at testing) offspring (mean + SEM). Paternal age had most significant effects on frequency of investigative behaviors ($p=0.0758$), as compared with frequencies of interaction-soliciting ($p=0.183$) and affiliative behaviors ($p=0.970$). Other parameters of investigative behaviors were also significantly affected by paternal age, with reductions in both total duration ($p=0.00684$) and single bout duration (0.039) in offspring of older breeders.

		Frequency (count)	Duration (s) (total)	Duration (s) (single bout)
Total social behaviors	**YF**	M=63.0 (5.87) M_M=56.7 (5.63); M_F=70.0 (10.60)	M=123.63 (11.43) M_M=135.54 (10.21) M_F=110.40 (21.20)	M=2.23 (0.213) M_M=2.82 (0.21) M_F=1.63 (0.26)
	OF	M=59.20 (5.43) M_M=54.3 (5.47) M_F=64.1 (9.44)	M=108.39 (10.98) M_M=118.14 (12.27) M_F=98.64 (18.36)	M=2.08 (0.23) M_M=2.44 (0.20) M_F=1.72 (0.39)
	VOF	M=59.4 (6.83) M_M=45.4 (4.31) M_F=73.4 (11.62)	M=104.74 (14.08) M_M=110.69 (15.88) M_F=98.79 (14.08)	M=2.15 (0.29) M_M=2.77 (0.49) M_F=1.54 (0.14)
	ANOVA p-values	(1) F_{PA} (1,56)=0.190, p_{PA}=0.664 F_{sex} (1,56)=6.598, p_{sex}=0.013*	(1) F_{PA} (1,56)=1.518, p_{PA}=0.223 F_{sex} (1,56)=2.298, p_{sex}=0.135	(1) F_{PA} (1,56)=0.104, p_{PA}=0.748 F_{sex} (1,56)=17.568, p_{sex}=9.9E-5**

		(2) $F(1,28)=2.456$, $p_M=0.128$ $F(1,27)=0.06$, $p_F=0.808$	(2) $F(1,28)=1.889$, $p_M=0.180$ $F(1,27)=0.206$, $p_F=0.654$	(2) $F(1,28)=0.009$, $p_M=0.924$ $F(1,27)=0.061$, $p_F=0.806$
	Levene's test	M: df(2,27), F=0.301, p=0.743 F: df(2,26), F=0.298; p=0.745	M: df(2,27), F=0.715, p=0.498 F: df(2,26), F=0.499 p=0.631	M: df(2,27), F=1.577, p=0.225 F: df(2,26), F=0.213 ; p=0.809
Investigative behaviors	**YF**	M=43.32 *(4.31* $M_M = 37.00$ *(3.16))* $M_F=50.33$ *(8.00)*	M=84.32 *(9.47)* $M_M=93.30$ *(13.05)* $M_F=74.34$ *(13.76)*	M=1.97 *(0.19)* $M_M=2.47$ *(0.25)* $M_F=1.42$ *(0.14)*
	OF	M=39.95 *(4.24)* $M_M=33.1$ *(2.87)* $M_F=46.80$ *(7.57)*	M=70.65 *(7.09)* $M_M=73.09$ *(9.19)* $M_F=68.22$ *(11.26)*	M=1.87 *(0.17)* $M_M=2.23$ *(0.26)* $M_F=1.51$ *(0.16)*
	VOF	M=42.25 *(5.39)* $M_M=29.6$ *(2.61)* $M_F=54.90$ *(8.97)*	M=63.95 *(6.81)* $M_M=52.59$ *(6.90)* $M_F=75.30$ *(10.94)*	M=1.59 *(0.11)* $M_M=1.76$ *(0.20)* $M_F=1.42$ *(0.09)*
	p-value	(1) F_{PA} (1,56)=0.028, $p_{PA}=0.867$; F_{sex} (1,56)=12.707, $p_{sex}=0.0008$** (2) F (1,28)=3.399, $p_M=0.0758$*; F (1,27(=0.17), $p_F=0.684$ Males pairwise comparisons: $p_{YF\text{-}OF}= 0.611$ $p_{YF\text{-}VOF} = 0.185$ $p_{OF\text{-}VOF} = 0.672$	(1) F_{PA} (1,56)=3.332, $p_{PA}=0.073$; F_{sex} (1,56)=0, $p_{sex}=0.993$ (2)F (1,28)=8.528, $p_M=0.00684$**; F (1,27)=0.005, $p_F=0.943$ Males pairwise comparisons: $p_{YF\text{-}OF}= 0.343$ $p_{YF\text{-}VOF} = 0.021$ $p_{OF\text{-}VOF} = 0.333$	(1) F_{PA} (1,56)=3.873, $p_{PA}=0.054$*; F_{sex} (1,56)=19.292, $p_{sex}=5.94E\text{-}5$** (2) F (1,28)=4.691, $p_M=0.039$*; F (1,27)=0.001, $p_F=0.98$ Males pairwise comparisons: $p_{YF\text{-}OF}= 0.754$ $p_{YF\text{-}VOF} = 0.102$ $p_{OF\text{-}VOF} = 0.348$
	Levene's test	M: df(2,27), F=0.154; p=0.858 F: df(2,26), F=0.225; p=0.800	M: df(2,27), F=1.230; p=0.308 F: df(2,26), F=0.0964; p=0.908	M: df(2,27), F=0.209; p=813 F: df(2,26), F=0.478; p=0.626
Affiliative behaviors	**YF**	M=12.16 *(2.08)* $M_M=11.20$ *(1.44)* $M_F=13.22$ *(4.20)*	M=37.15 *(8.10)* $M_M=40.20$ *(8.48)* $M_F=33.76$ *(14.80)*	M=3.06 *(0.54)* $M_M=3.83$ *(0.77)* $M_F=2.09$ *(0.63)*
	OF	M=12.80 *(1.69)* $M_M=14.50$ *(2.21)* $M_F=11.10$ *(2.56)*	M=33.79 *(7.38)* $M_M=43.18$ *(8.20)* $M_F=24.41$ *(11.98)*	M=2.35 *(0.35)* $M_M=2.97$ *(0.34)* $M_F=1.72$ *(0.56)*
	VOF	M=10.60 *(1.64)* $M_M=11.10$ *(1.82)* $M_F=10.10$ *(2.82)*	M=39.90 *(10.23)* $M_M=57.31$ *(17.63)* $M_F=22.50$ *(7.97)*	M=2.96 *(0.51)* $M_M=4.24$ *(0.81)* $M_F=1.62$ *(0.31)*
	p-value	(1) F_{PA} (1,56)=0.380, $p_{PA}=0.540$; F_{sex} (1,56)=0.156, $p_{sex}=0.694$ (2) F (1,28)=0.001, $p_M=0.970$; F (1,27)=0.478, $p_F=0.49$	(1) F_{PA} (1,56)=0.057, $p_{PA}=0.812$; F_{sex} (1,56)=4.410, $p_{sex}=0.0402$ (2) F(1,28)=1.007, $p_M=0.324$; F(1,27)=0.459, $p_F=0.504$	(1) F_{PA} (1,55)=0.014, $p_{PA}=0.905$; F_{sex} (1,55)=14,544, $p_{sex}=3.48E\text{-}4$ (2) F(1,28)=0.182, $p_M=0.673$; F(1,26)=0.308, $p_F=0.583$

	Levene's test	M: df(2,27), F=0.689; p=0.511 F: df(2,26), F=0.538; p=0.590	M: df(2,27), F=1.428; p=0.257 F: df(2,26), F=0.384; p=0.685	M: df(2,27), F=1.316; p=0.285 F: df(2,25), F=0.523; p=0.599
Interaction-soliciting behaviors	**YF**	M=7.53 *(1.30)* M_M=8.50 *(2.22)* M_F=6.44 *(1.27)*	-	-
	OF	M=6.45 *(1.13)* M_M=6.70 *(1.75)* M_F=6.20 *(1.53)*	-	-
	VOF	M=6.55 *(2.01)* M_M=4.70 *(2.01)* M_F=8.40 *(3.50)*	-	-
	p-value	(1) F_{PA} (1,56)=0.195, p_{PA}=0.660; F_{sex} (1,56)=0.055, p_{sex}=0.815 (2) F(1,28)=1.864, p_M=0.183; F(1,27)=0.356, p_F=0.556	-	-
	Levene's test	M: df(2,10), F=0.202; p=0.819 F: df(2,12), F=0.424; p=0.659	-	-
Inactive	**YF**	M=1.16 *(0.33)* M_M=1.10 *(0.38)* M_F=1.22 *(0.57)*	M=2.16 *(0.64)* M_M=2.04 *(0.86)* M_F=2.30 *(1.0)*	M=1.87 *(0.35)* M_M=1.75 *(0.54)* M_F=2.08 *(0.35)*
	OF	M=1.95 *(0.59)* M_M=1.30 *(0.72)* M_F=2.60 *(0.92)*	M=3.94 *(1.47)* M_M=1.87 *(0.99)* M_F=6.02 (*2.69*)	M=2.29 *(0.99)* M_M=1.50 *(0.39)* M_F=2.75 *(1.56)*
	VOF	M=0.65 *(0.24)* M_M=0.30 *(0.21)* M_F=1.00 *(0.42)*	M=0.89 *(0.34)* M_M=0.79 *(0.54)* M_F=0.98 *(0.43)*	M=1.67 *(0.44)* M_M=2.83 *(0.55)* M_F=1.09 *(0.30)*
	p-value	(1) F_{PA} (1,56)=0.768, p_{PA}=0.385; F_{sex} (1,56)=2,237, p_{sex}=0.140 (2) F(1,28)=1.368, p_M=0.252; F(1,27)=0.075, p_F=0.782	(1) F_{PA} (1,56)=0.905, p_{PA}=0.346; F_{sex} (1,56)=1.975, p_{sex}=0.165 (2) F(1,28)=1.194, Fp_M=0.284; F(1,27)=0.323, p_F=0.575	(1) F_{PA} (1,25)=0.005, p_{PA}=0.947; F_{sex} (1,25)=0.133, p_{sex}=0.719 (2) F(1,11)=0.649, p_M=0.438; F(1,13)=0.233, p_F=0.637
	Levene's test	M: df(2,27), F=1.173; p=0.325 F: df(2,26), F=1.320; p=0.285	M: df(2,27), F=0.592; p=0.560 F: df(2,26), F=2.325; p=0.118	M: df(2,27), F=0.273; p=0.767 F: df(2,26), F=0.466; p=0.639

Table 3. Descriptive (mean and standard error in brackets), ANOVA and Levene's tests statistics for social investigation parameters, total social behaviors and grouped by interaction type. For interaction-soliciting items only frequency measures could be reliably determined; 'inactive' was the only item in this domain where durations could be computed, and statistics are presented in the table. ANOVA test was used for all of the comparisons. P-values are presented under a model where both sex and PA were entered as factors (1), and where sexes were analyzed separately, with PA as the only factor (2). For all significant results post-hoc analyses were run (Tukey HSD test), and pairwise group comparisons reported. Levene's test was run to determine if there were any significant differences between the groups' variances.

Discussion:

Deficits in social functions have been reported in previous APA mouse models (Sampino et al., 2014; Smith et al., 2009). Our design allowed us to further confirm the *de novo* origins in the offspring generation, and have revealed a sexually dimorphic developmental trajectory for social behavior.

Our study represents an important contribution to APA research as it provides the first experimental evidence that altered behavior in the offspring generation does not arise as a result of differences in the behavior of their fathers. Our study strengthens the claim that some of the age-related DNA changes, previously observed in the sperm of aged men, are indeed causative in the association between APA and psychiatric disorders in the offspring (Flatscher-Bader et al., 2011; Kong et al., 2012; Milekic et al., 2014). Although we cannot rule out a potential influence of maternal behavior on the offspring, all dams were of the same age and inbred strain; furthermore, male breeders, in which no group differences in social behavior were observed, were removed 7 days prior to littering down so differences in maternal behavior were likely to be minimal.

In the context of no differences in paternal social behavior, juvenile offspring fathered by aged males showed increased levels of sociability relative to those fathered by young males; these differences were recorded across all behavior types. This is in parallel with high levels of social interaction in some individuals with ASD, whose social behaviors may nevertheless be seen as excessive or inappropriate (Matson & Wilkins, 2007; Rumsey, Rapoport, & Sceery, 1985), "active but odd" (Wing, 1981). Although items describing social disinhibition feature in standard autism assessment instruments (ADOS-G (Lord et al., 1989), ADI-R

(Lord, Rutter, & Le Couteur, 1994)), to the best of our knowledge there is currently no research on its life-long consequences in humans.

In our study, as the animals progressed into adulthood, the social profile significantly changed for males, especially for the investigative behaviors, for which frequency, total duration and duration of each interactive bout were lower in the offspring of aged breeders. These observations resemble social withdrawal, often associated with ASD.

Having conducted a comprehensive battery of behavioral tests, we have high confidence that our results are not due to other behavioral confounders. Differences in the sociability could not be explained by APA-related olfactory deficit (all animals show normal olfactory habituation/dishabituation profiles), nor by differential sensitivity to stress (no group differences in the open field test). We observed a mild deficit in motor co-ordination in male VOF offspring, further reinforcing validity of our model to neurodevelopmental disorders (Fournier, Hass, Naik, Lodha, & Cauraugh, 2010; Schwartz, Rosse, Veazey, & Deutsch, 1996). However, this motor deficit was mild and transient; there were no differences in exploratory or locomotor activity as assessed in the holeboard and homecage test, removing further confounds from our assessment of their sociability. Discussing our data, one has to account both for (i) the developmental effects on social profile in males, and (ii) lack of persistence of these effects in adult females. It remains to be elucidated whether the developmental changes observed by us happened as a result of social experiences or experience-independent factors. Other models of neurodevelopmental disorders also suggest that neurodevelopmental disruption early in life can have a long-lasting impact on sociability in rodents, and that these effects are likely to fluctuate across time. Schneider and Koch (2005) showed that lesions in the medial prefrontal cortex resulted in a decrease in social behaviors in both juvenile and adult rats, and these effects coincided with an increase in

social exploration in adulthood (Schneider & Koch, 2005). In our study, (epi)genetic disruption arising due to APA could have a similarly adverse impact on neurodevelopment in Generation 1 mice. Normal developmental trajectories are guided by the dynamic regulation of gene expression, which is tightly regulated by epigenomic processes (Jaffe, Gao, & Tao, 2014; Numata et al., 2012). Failure to retain typical epigenetic temporal patterns could account for the derailment of social development, and persistence of the APA effects in male offspring.

It is possible that females, in spite of being affected by APA to the same degree as males when juvenile, are better at developing compensatory mechanisms such that overt alterations in the social behavior domain do not persist into adulthood. This explanation is in line with observations from humans, reporting that overall females show higher degrees of compensatory mechanisms (Lai et al., 2011), and require a greater genetic burden before displaying behavioral symptoms (Jacquemont et al., 2014; Robinson, Lichtenstein, Anckarsäter, Happé, & Ronald, 2013). The nature of this 'female protective effect' remains elusive (Gockley et al., 2015; Lai, Baron-Cohen, & Buxbaum, 2015). We cannot rule out effects of sex hormones, which have been suggested to play a role in sexually dimorphic disease presentation in humans (Markham, 2012; Werling & Geschwind, 2013), and whose role is likely to become more prominent later in life.

Our study adds important insights to our understanding of the APA effects, validating the previous epidemiological findings and suggesting APA could underpin a distinct social profile in neurodevelopmental disorders. Further research is warranted to explore other ASD-related behavioral domains (e.g. communication detected via ultrasonic vocalization and stereotypies and repetitive behavior), which could shed additional light on whether APA is associated with unique disease subtypes, a possibility that has been suggested before

(Malaspina et al., 2012). However, it is unlikely that a single animal model would recapitulate deficits in all of the behavioral domains implicated in autism.

In our study the choice of age of the oldest paternal group was based on our original study (Smith et al., 2009) and driven by concerns of decreasing fertility when using male mice older than 12 months. Age of the oldest breeders in our study approximated to around 40 years of age in humans. Epidemiological findings suggest that odds ratio for having a child with autism is already increased in men aged 30-39 (OR = 1.64), and rises even higher for men in 40-49 (OR = 5.65) and 50-59 (OR = 9.39) age categories (Reichenberg et al., 2006). Although the odds ratio estimates differ between studies, there seems to be a consensus about higher risk for offspring neurodevelopmental disorders starting already in men in their late 30s (Croen, Najjar, Fireman, & Grether, 2007; Malaspina, 2001; Tsuchiya et al., 2008). Furthermore, relatively few men have children beyond their late 40s so studying APA effects in an upper but not extreme age range is of greater epidemiological relevance. Nevertheless, some studies have used extended paternal age groups (12-18 months (Foldi et al., 2010)) and these approaches could provide insights into the dynamics of the effects of APA on offspring (plateauing at some stage vs further linear/exponential increase). Future research could focus on these very old breeders as long as sufficient numbers of breeders are set-up given the reduced fecundity of very old males.

The disorders most commonly associated with APA (autism and schizophrenia), are widely considered to be neurodevelopmental in nature, likely resulting from cascades of events during development that are triggered by an insult preceding the emergence of a full-blown phenotype (Rapoport, Giedd, & Gogtay, 2012). In our animal model, we observed dynamic and long-lasting behavioral consequences of a prenatal factor (APA), thus supporting the notion that APA effects operate through their influence on early development. Our results

suggest a strong translational point that there is a critical need for studies examining social development in children with autism who exhibit excessive sociability early in their lives, and factors that could influence their developmental trajectory.

Acknowledgements:

M.J. was sponsored by the Medical Research Council PhD studentship (MR/J500380/1). All animal costs were covered by the grant from the Icahn Medical School at Mount Sinai, "Trans-Generational Study of the Effects of Sex and Development Stage on the Behavior of the Offspring of Aged Fathers" (ref: 0285-3965-4609). All reported data is stored at King's College London and available upon request. The authors do not report any conflicts of interest. Authors' contributions: study design: A.R., C.F., J.M., L.S., M.J.; behavioral testing: M.J.; data scoring: M.S., A.M., V.T.; data analysis: M.J.; manuscript writing: M.J.; manuscript revisions: C.F., M.S., A.M., V.T., J.M., L.S., A.R., R.S.

We thank Robert Plomin for critically reading the manuscript.

Conflict of interest:

The authors declare no conflict of interest.

References:

Croen, L., Najjar, D. V., Fireman, B., & Grether, J. K. (2007). Maternal and paternal age and risk of autism spectrum disorders. *Archives of Pediatrics & Adolescent Medicine*, *161*(4), 334–40. doi:10.1001/archpedi.161.4.334

D'Onofrio, B. M., Rickert, M. E., Frans, E., Kuja-Halkola, R., Almqvist, C., Sjölander, A., … Lichtenstein, P. (2014). Paternal age at childbearing and offspring psychiatric and academic morbidity. *JAMA Psychiatry*, *71*(4), 432–8. doi:10.1001/jamapsychiatry.2013.4525

Flatscher-Bader, T., Foldi, C. J., Chong, S., Whitelaw, E., Moser, R. J., Burne, T. H. J., … McGrath, J. J. (2011). Increased de novo copy number variants in the offspring of older males. *Translational Psychiatry*, *1*(8), e34. doi:10.1038/tp.2011.30

Foldi, C. J., Eyles, D. W., McGrath, J. J., & Burne, T. H. J. (2010). Advanced paternal age is associated with alterations in discrete behavioural domains and cortical neuroanatomy of C57BL/6J mice. *The European Journal of Neuroscience*, *31*(3), 556–64. doi:10.1111/j.1460-9568.2010.07074.x

Fournier, K., Hass, C. J., Naik, S. K., Lodha, N., & Cauraugh, J. H. (2010). Motor coordination in autism spectrum disorders: a synthesis and meta-analysis. *Journal of Autism and Developmental Disorders*, *40*(10), 1227–40. doi:10.1007/s10803-010-0981-3

Frans, E. M., Sandin, S., Reichenberg, A., Lichtenstein, P., Långström, N., & Hultman, C. M. (2008). Advancing paternal age and bipolar disorder. *Archives of General Psychiatry*, *65*(9), 1034–40. doi:10.1001/archpsyc.65.9.1034

Gockley, J., Willsey, A. J., Dong, S., Dougherty, J. D., Constantino, J. N., & Sanders, S. J. (2015). The female protective effect in autism spectrum disorder is not mediated by a single genetic locus. *Molecular Autism*, *6*(1), 1–10. doi:10.1186/s13229-015-0014-3

Jacquemont, S., Coe, B. P., Hersch, M., Duyzend, M. H., Krumm, N., Bergmann, S., … Eichler, E. E. (2014). A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *American Journal of Human Genetics*, *94*(3), 415–25. doi:10.1016/j.ajhg.2014.02.001

Jaffe, A. E., Gao, Y., & Tao, R. (2014). The methylome of the human frontal cortex across development. doi: http://dx.doi.org/10.1101/005504

Kong, A., Frigge, M. L., Masson, G., Besenbacher, S., Sulem, P., Magnusson, G., … Stefansson, K. (2012). Rate of de novo mutations and the importance of father's age to disease risk. *Nature*, *488*(7412), 471–5. doi:10.1038/nature11396

Lad, H. V., Liu, L., Paya-Cano, J. L., Parsons, M. J., Kember, R., Fernandes, C., & Schalkwyk, L. C. (2010). Behavioural battery testing: evaluation and behavioural outcomes in 8 inbred mouse strains. *Physiology & Behavior*, *99*(3), 301–16. doi:10.1016/j.physbeh.2009.11.007

Lai, M.-C., Baron-Cohen, S., & Buxbaum, J. D. (2015). Understanding autism in the light of sex/gender. *Molecular Autism*, *6*(1), 1–5. doi:10.1186/s13229-015-0021-4

Lai, M.-C., Lombardo, M. V, Pasco, G., Ruigrok, A. N. V, Wheelwright, S. J., Sadek, S. A., … Baron-Cohen, S. (2011). A behavioral comparison of male and female adults with high functioning autism spectrum conditions. *PloS One*, *6*(6), e20835. doi:10.1371/journal.pone.0020835

Life span as a biomarker. (2011). *The Jackson Laboratory*. Retrieved from http://research.jax.org/faculty/harrison/ger1vLifespan1.html#VD

Lord, C., Rutter, M. L., Goode, S., Heemsbergen, J., Jordan, H., Mawhood, L., & Schopler, E. (1989). Autism Diagnostic Observation Schedule: A Standardized Observation of Communicative and Social Behavior. *Journal of Autism and Developmental Disorders*, *19*(2), 185–212.

Lord, C., Rutter, M., & Le Couteur, A. (1994). Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders*, *24*(5), 659–85.

Malaspina, D. (2001). Paternal factors and schizophrenia risk: de novo mutations and imprinting. *Schizophrenia Bulletin*, *27*(3), 379–93.

Malaspina, D., Keller, A., Antonius, D., Perrin, M., Opler, M., Ahn, H., … Harlap, S. (2012). Advanced paternal age contributes to a specific subtype of schizophrenia. *Schizophrenia Research*, *136*(1), S3.

Markham, J. (2012). Sex steroids and schizophrenia. *Reviews in Endocrine & Metabolic Disorders*, *13*(3), 187–207. doi:10.1007/s11154-011-9184-2

Matson, J. L., & Wilkins, J. (2007). A critical review of assessment targets and methods for social skills excesses and deficits for children with autism spectrum disorders. *Research in Autism Spectrum Disorders*, *1*(1), 28–37. doi:10.1016/j.rasd.2006.07.003

Milekic, M. H., Xin, Y., O'Donnell, A., Kumar, K. K., Bradley-Moore, M., Malaspina, D., … Gingrich, J. A. (2014). Age-related sperm DNA methylation changes are transmitted to offspring and associated with abnormal behavior and dysregulated gene expression. *Molecular Psychiatry*, (January), 1–7. doi:10.1038/mp.2014.84

Mill, J., Galsworthy, M. J., Paya-Cano, J. L., Sluyter, F., Schalkwyk, L. C., Plomin, R., & Asherson, P. (2002). Home-cage activity in heterogeneous stock (HS) mice as a model of baseline activity. *Genes, Brain, and Behavior*, *1*(3), 166–73.

Miller, B., Suvisaari, J., Miettunen, J., Järvelin, M.-R., Haukka, J., Tanskanen, A., … Kirkpatrick, B. (2011). Advanced paternal age and parental history of schizophrenia. *Schizophrenia Research*, *133*(1-3), 125–32. doi:10.1016/j.schres.2011.08.013

Numata, S., Ye, T., Hyde, T. M., Guitart-Navarro, X., Tao, R., Wininger, M., … Lipska, B. K. (2012). DNA methylation signatures in development and aging of the human prefrontal cortex. *American Journal of Human Genetics*, *90*(2), 260–72. doi:10.1016/j.ajhg.2011.12.020

Perrin, M. C., Brown, A. S., & Malaspina, D. (2007). Aberrant epigenetic regulation could explain the relationship of paternal age to schizophrenia. *Schizophrenia Bulletin*, *33*(6), 1270–3. doi:10.1093/schbul/sbm093

Petersen, L., Mortensen, P. B., & Pedersen, C. B. (2011). Paternal age at birth of first child and risk of schizophrenia. *The American Journal of Psychiatry*, *168*(1), 82–8. doi:10.1176/appi.ajp.2010.10020252

Rapoport, J. L., Giedd, J. N., & Gogtay, N. (2012). Neurodevelopmental model of schizophrenia : update 2012. *Molecular Psychiatry*, *17*(12), 1228–1238. doi:10.1038/mp.2012.23

Reichenberg, A., Gross, R., Weiser, M., Bresnahan, M., Silverman, J., Harlap, S., … Susser, E. (2006). Advancing paternal age and autism. *Archives of General Psychiatry*, *63*(9), 1026–32. doi:10.1001/archpsyc.63.9.1026

Robinson, E. B., Lichtenstein, P., Anckarsäter, H., Happé, F., & Ronald, A. (2013). Examining and interpreting the female protective effect against autistic behavior. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(13), 5258–62. doi:10.1073/pnas.1211070110

Rumsey, J., Rapoport, J., & Sceery, W. (1985). Autistic Children as Adults: Psychiatric, Social and Behavioral Outcomes. *Journal of American Academy of Child Psychiatry*, *24*(4), 465–473.

Sampino, S., Juszczak, G. R., Zacchini, F., Swiergiel, A. H., Modlinski, J. a, Loi, P., & Ptak, G. E. (2014). Grand-paternal age and the development of autism-like symptoms in mice progeny. *Translational Psychiatry*, *4*(4), e386. doi:10.1038/tp.2014.27

Schneider, M., & Koch, M. (2005). Behavioral and morphological alterations following neonatal excitotoxic lesions of the medial prefrontal cortex in rats. *Experimental Neurology*, *195*(1), 185–198. doi:10.1016/j.expneurol.2005.04.014

Schwartz, B. L., Rosse, R. B., Veazey, C., & Deutsch, S. I. (1996). Impaired motor skill learning in schizophrenia: Implications for corticostriatal dysfunction. *Biological Psychiatry*, *39*(4), 241–248. doi:10.1016/0006-3223(95)00130-1

Smith, R. G., Kember, R. L., Mill, J., Fernandes, C., Schalkwyk, L. C., Buxbaum, J. D., & Reichenberg, A. (2009). Advancing paternal age is associated with deficits in social and exploratory behaviors in the offspring: a mouse model. *PloS One*, *4*(12), e8456. doi:10.1371/journal.pone.0008456

Smith, R. G., Reichenberg, A., Kember, R. L., Buxbaum, J. D., Schalkwyk, L. C., Fernandes, C., & Mill, J. (2012). Advanced paternal age is associated with altered DNA methylation at brain-expressed imprinted loci in inbred mice: implications for neuropsychiatric disease. *Molecular Psychiatry*, 1–2. doi:10.1038/mp.2012.88

Terranova, L., & Laviola, G. (2005). Scoring of Social Interactions and Play in Mice During Adolescence. In *Current protocols in toxicology* (p. Unit 13.10).

Tsuchiya, K. J., Matsumoto, K., Miyachi, T., Tsujii, M., Nakamura, K., Takagai, S., … Takei, N. (2008). Paternal age at birth and high-functioning autistic-spectrum disorder in offspring. *The British Journal of Psychiatry : The Journal of Mental Science*, *193*(4), 316–21. doi:10.1192/bjp.bp.107.045120

Werling, D. M., & Geschwind, D. H. (2013). Sex differences in autism spectrum disorders. *Current Opinion in Neurology*, *26*(2), 146–53. doi:10.1097/WCO.0b013e32835ee548

Wing, L. (1981). Language, Social and Cognitive Impairments in Autism and Severe Mental Retardation. *Journal of Autism and Developmental Disorders*, *11*(1), 31–44.

Winslow, J. T. (2003). Mouse social recognition and preference. *Current Protocols in Neuroscience / Editorial Board, Jacqueline N. Crawley ... [et Al.]*, *Chapter 8*, Unit 8.16. doi:10.1002/0471142301.ns0816s22