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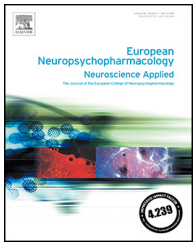

Inhibitory control in BALB/c mice sub-strains during extinction learning

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Abstract
Dysregulation of executive function (EF) involves alterations in cognitive flexibility / control and is underscored by learning impairments in neurodevelopmental disorders. Here, we examine cognitive inflexibility in BALB/cJ mice (a mouse model showing diminished sociability, increased anxiety and inattentive behaviour) and closely related "reference" BALB/cByJ mice. We used an appetitive extinction paradigm to investigate if cognitive flexibility measures are different between learning acquisition and extinction. The two BALB/c sub-strains learned to respond to a stimulus in a touchscreen operant chamber, after which the reward was removed and responses should be inhibited. Both mice sub-strains showed a different rate of learning while acquiring the task, in which the BALB/cJ mice were faster learners compared to the BALB/cByJ mice. This was not observed during the extinction phase, in which the BALB/cJ mice were able to extinguish responding to unrewarded stimuli equally. Within the BALB/cJ sub-strain, variation in the ability to inhibit a learnt response was observed when comparing them to similar grouped BALB/cByJ mice: BALB/cJ animals that reached the criterion were more reward driven, while BALB/cJ mice failing to reach the set criterion during extinction processing make more mistakes. Additionally, the changes observed during acquisition, were driven by animals not reaching the extinction criterion. Our results suggest that the BALB/c mice sub-strains may use different strategies to learn during appetitive extinction. This may be useful in the phenotypic dissection of cognitive flexibility in BALB/c sub-strains and their mapping on genetic variance revealed by next-generation sequencing in future studies.

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1. Introduction

Executive function (EF) comprises a set of cognitive control processes, mainly supported by the prefrontal cortex, which regulates lower level processes (e.g., perception, motor responses). It enables self-regulation and self-directed behaviour toward a goal, allowing us to select, update and plan motor sequences, withholding and/or stopping of actions (Craig, 2016). Furthermore it is also involved in monitoring and changing behaviour where appropriate; and dividing, switching and sustaining attention (Miyake and Friedman, 2012). Dysfunctions in EF are associated with a variety of psychiatric and neurological disorders and therefore has been shown to be a promising neuropsychological trait for the understanding of the pathophysiology of neurodevelopmental disorders, such as autism spectrum disorder (ASD) and attention-deficit hyperactivity disorder (ADHD)(Craig, 2016). While the core symptoms of these disorders seem very different, ADHD and ASD share overlapping features that co-occur. This co-occurrence of symptoms is supported by clinical, epidemiological, genetic, neurobiological, and environmental risk factors i.e. prenatal exposures, familial history and environmental contaminants (Lamanna, 2017; Martin, 2014; Musser, 2014; Pinto, 2016; Rommelse, 2010, 2011; Tran and Miyake, 2017). Moreover, ASD and ADHD also share a dysfunction of top-down inhibitory control regulating impulsivity and compulsivity (Berlin and Hollander, 2014; Fineberg, 2010; Naaijen, 2015; Robbins, 2012; Rommelse, 2010, 2011).

Studying inhibitory control has the advantage of dealing with a relatively simple and straightforward process: the over-riding of a planned or already initiated action (Bari and Robbins, 2013). Deficient inhibitory processes profoundly affect everyday life, causing cognitive inflexibility/rigidity which is generally detrimental for the individual as seen in patients with the above mentioned disorders. For example, failing to inhibit one's urge to physically harm another person, is not only detrimental for oneself (i.e. one might end up in prison) but also for the other person (i.e. sustaining injuries). One way to investigate deficiencies in inhibitory control and cognitive flexibility in a laboratory setting is the use of an extinction paradigm (Bouton, 2004). In principle, during the task a behaviour that has previously been reinforced will no longer be reinforced. This procedure makes the behaviour ineffective such that it should occur less and less until it eventually stops altogether (complete inhibition)(Cooper et al., 2007; Mar, 2013; Miltenberger, 2008).

Animal models not only allow for investigating cognitive flexibility, but also the underlying neural substrates. A number of animal models, especially mouse and rat models, have been tested on cognitive flexibility with discrete differences reported (Bari and Robbins, 2013). Extending the paradigm to rodents, one can use the appetitive extinction paradigm, which includes an acquisition phase in which the rodents are trained to respond to a stimulus followed by the extinction phase, where the reward will be stopped and inhibition of the behaviour should appear (Mar, 2013). The speed and degree of inhibition of the learnt response over time is a measure of rigidity (Mar, 2013; Quirk and Mueller, 2008). It can also provide insight in the decision strategies used to learn and stop responding to the stimulus. An interesting model to study this extinction behaviour are the two BALB/c inbred mice sub-strains: the BALB/cJ and BALB/cByJ. These genetically related but phenotypically distinct sub-strains show behavioural profiles that resemble ASD and ADHD symptoms (e.g. social withdrawal and repetitive behaviour (Argyropoulos et al., 2013; Brodkin, 2007; Burket, 2016; Fairless, 2008, 2013; Jager, 2017). The degree of these behavioural abnormalities differs, however, between the sub-strains, namely: BALB/cJ mice show higher levels of anxiety (Brodkin, 2007; Fairless, 2013; Zarcone et al., 2004) and aggression (Dow et al. 2011; Jager, 2017; Velez, 2010), and lower social approach and direct interaction (Brodkin, 2004,2007; Fairless, 2008; Jacome, 2011; Moy, 2007; Sankoorikal, 2006). BALB/cJ mice are more reward focused and unable to learn from mistakes during a visual discrimination task compared to the BALB/cByJ mice (Graybeal, 2014; Jager, 2019). In addition, BALB/cJ mice demonstrate clear within sub-strain differences in sociability and empathy-like behaviour (Laviola, 2017). While the mentioned stereotypy and performances on learning in cognitive tasks have been documented for BALB/c sub-strains, data on extinction behaviour during an appetitive extinction learning task in these sub-strains has not yet been characterised.

The touchscreen extinction assay used in this study utilises an appetitive extinction paradigm from the computerised Cambridge Neuropsychological Test Automated Battery (CANTAB). The CANTAB utilizes a touchscreen computer interface rodent paradigm (Horner, 2013; Mar, 2013; Oomen, 2013) that translates directly to well established monkey and human touch models (Bussey, 2012; Talpos, 2009). The mechanisms behind extinction are intricate, its expression presumably contains numerous factors and underlying processes (e.g., generalization decrement, response inhibition, Pavlovian and/ or instrumental learning mechanisms) (Lattal and Lattal, 2012), and the interpretation of the results is complex. Care must be taken as performance during the task has been noted to be highly dependent on context, making an animal's distant and recent learning history influencing the learning and 'unlearning' (Mar, 2013). The ability to learn may underlie deficits in EF measures. In the context of appetitive extinction, this is relevant to both the task acquisition and the ability to suppress a previously learnt response. The investigation of learning ability in influencing BALB/c extinction performance may be useful to add to the fuller understanding of the behavioural repertoire within and between these sub-strains.

In the current study, we sought to compare two BALB/c sub-strains (BALB/cJ and BALB/cByJ mice) as it is clear that these differ in their social, anxiety and aggressive phenotype. The ability to learn, process, implement and change rules is dependent on rule-learning based strategies and requires cognitive flexibility. Here, we investigated differential effects in these substrains using a reward based rule-learning task called visual appetitive extinction learning in operant touchscreens, which also assesses the ability to inhibit non-rewarded behaviour. We previously observed phenotypical differences within BALB/cJ and BALB/cByJ sub-strains (Jager, 2017), and hypothesised that changes in the ability to learn and inhibit rules may in part contribute to within-strain behavioural differences. Therefore, we also assessed task performance in appetitive extinction between

animals that successfully reached the learning criterion and those that did not during the extinction phase.

2. Experimental procedures

2.1. Subjects

Thirty two male animals, sixteen BALB/cJ and sixteen BALB/cByJ mice (The Jackson Laboratory, USA), were trained to perform the appetitive extinction paradigm, of which five died before finishing the experiment (four BALB/cJ, one BALB/cByJ). Prior to the extinction paradigm, the animals performed a reversal learning with and without punishment during the task. This prior experience did not influence the outcome of the extinction paradigm, demonstrated by equal performance between BALB/c sub-strains at the start of the acquisition phase (**Supplementary Fig. 1**). The mice were 8 months old and between 23.5 and 29 g body weight when starting the appetitive extinction paradigm. Their weight was maintained at 90–95% of the free-feeding weight throughout the experiment in order to provide a motivational stimulus to work for the liquid food reward used in operant conditioning. Water was available *ad libitum* and corncob bedding (Bio Services B.V. Uden; The Netherlands) was provided including some nesting material and an igloo as enrichment. Inside a scantainer, the animals were housed individually (Scanbur Technology, Karlslunde, Denmark) under a 12 h/12 h reversed light-dark cycle (lights off at 07:30 h; lights on at 19:30 h). The temperature was controlled and maintained at +/- 23 °C. The experiments reported herein were performed in compliance with the ethical guidelines of the Dutch Ministry of Agriculture (Dutch Ethical Committee (DEC) licence number 2013-235).

2.2. Behavioural apparatus and training

2.2.1. Apparatus

Behavioural testing for the appetitive extinction learning was conducted in eight Bussey–Saksida mouse touchscreen operant chambers enclosed with Sound Attenuation Cubicles (SAC) (Campden Instruments Ltd., United Kingdom (UK)). The Bussey–Saksida chamber has a unique trapezoidal wall shape to focus the animal's attention towards the touchscreen. In front of the touch screen a mask was positioned with three holes. The reward tray, positioned 2 cm above the bar floor located opposite to the response holes, was attached to an external food dispenser equipped to deliver a drop of strawberry milkshake (20 μl) to the magazine. A stimulus was shown on the screen at the back of the middle mask hole. Also, a light was situated within the food magazine. Nosepoke responses onto the screen and food magazine aperture were detected by a horizontal infrared beam. Chambers could be illuminated by a houselight, and were controlled by WhiskerServer® and ABET II software from Lafayette Instruments neuroscience. Both the chambers and programs used in these experiments originate from Prof. Bussey and Dr. Saksida, Dept. of Experimental Psychology, University of Cambridge, UK and licenced to Campden Instruments Ltd., UK.

2.3. Appetitive extinction learning (AEL)

2.3.1. Acquisition phase

The touchscreen task performed by the animals is the appetitive extinction task (Mar, 2013). Animals were first habituated to the touchscreen box as described in the mentioned article. Then the animals were trained to acquire a simple visually guided response in order to earn a reward (Fig. 1(A)). During this response acquisition phase, sessions began with a free reward delivery to the magazine together with magazine light illumination, indicating that a trial may be initiated. Trials were initiated by the animal's head entry into the magazine (turning off the magazine light and activating a 0.2-s auditory click), in which subsequent head withdrawal from the magazine initiates presentation of a single, solid white square stimulus at the central hole on the touchscreen mask. When the animal touched the stimulus (response), this was removed from the screen, a reward was delivered and the magazine light and a 1-s tone were turned on. Following reward collection, the magazine light was turned off and a 5-s Inter-Trial-Interval (ITI) commences after which a new trial begins. When the animals reached a criterion of 30 responses out of 30 trials (100% responses) in 12.5 min for five consecutive days, the animals started the extinction phase.

2.3.2. Extinction phase

In the extinction phase, each session contained 30 trials. Each trial began with a 10-s ITI, after which the single, solid white square stimulus was presented on the touchscreen (same shape and size as in the acquisition phase; Fig. 1(B)). The animal did not have to initiate the trial by entering its head into the magazine. The stimulus was removed either when the animal touched the stimulus (response) or did not touch the stimulus within a 10-s duration (omission). No rewards or conditioned reinforcers (e.g., tray light or tone associated with reward delivery) were delivered during this extinction phase. In this phase the animal was reaching criterion when having ≥23 omissions out of the 30 trials (≥75% omissions) for two consecutive days. All animals performed 15 sessions of extinction.

2.4. Measures

The primary measures for the acquisition and extinction phase are (i) the number of sessions and responses to reach criterion which provide information on the ability to learn and 'unlearn' or inhibit responding, respectively (Mar, 2013). In addition, (ii) the number of blank touches required to reach criterion and (iii) mean response rate were examined to provide information on the learning in the acquisition phase and the amount of inhibition, and therefore the level of flexibility in the extinction phase. Three latencies were included as an index of reward sensitivity and motivation, which likely interacts with rigid behaviour. These latencies are: (iv) the time to respond to the stimulus (**response latency**), v) the time to touch the screen where no stimulus was presented (**blank touch latency**), (vi) the time to check the tray for the reward (acquisition phase) or check the food magazine without reward being delivered (extinction phase) (**reward latency**). For each of these latencies the data of the first and final day of acquisition and extinction were compared as well within and between the two strains to gain insight in the changes over time in the reward sensitivity and motivation.

For analysis of the acquisition and extinction phase of the appetitive extinction paradigm, sessions were analysed (a) within and between sub-strains and (b) within and between the BALB/c sub-strains that are able to reach (>75% omissions or <25% responses) and not reach (<75% omissions) the criterion during the extinction phase. Additionally, these subgroups from the extinction phase (reaching and not-reaching) were used to analyse data from the acquisition phase to assess possible effects of the learning ability on extinction (see **Supplementary Table 4, Supplementary results and Supplementary Figs. 6 and 7**). This was examined both between and within the subgroups and sub-strains.

2.5. Statistical analysis

All outcome measures were checked for normality using the Shapiro-Wilks' test and a visual inspection of their boxplots. The normally distributed variables were analysed using independent and dependent Student *t-test*. Not normally distributed data were analysed with non-parametric Mann-Whitney U-tests (independent samples) and Wilcoxon Signed Ranks Tests (paired-samples).

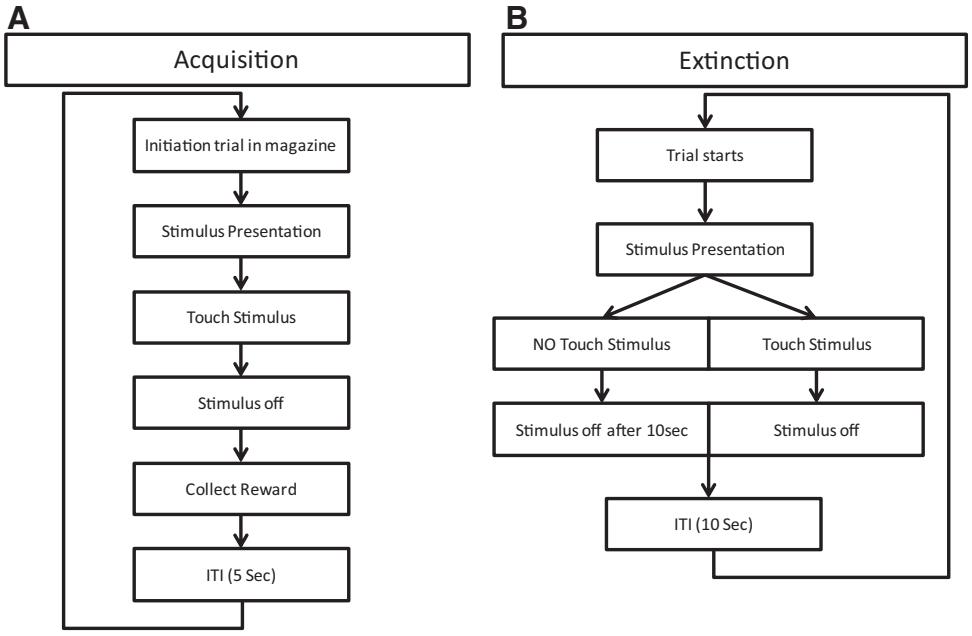

Fig. 1 Flowchart of the acquisition (A) and extinction phase (B). (A) In the acquisition phase the animal needs to initiate the trial and when touching the stimulus a reward is provided. Picking up the reward will lead to an inter-trial-interval (ITI) of 5 s before the next trial can be initiated. (B) In contrast, during the extinction phase the trials start automatic and when the animal is touching the stimulus there is no reward provided. Instead an ITI of 10 s will start before the next trial starts. Created based on the protocol used from (Mar, 2013).

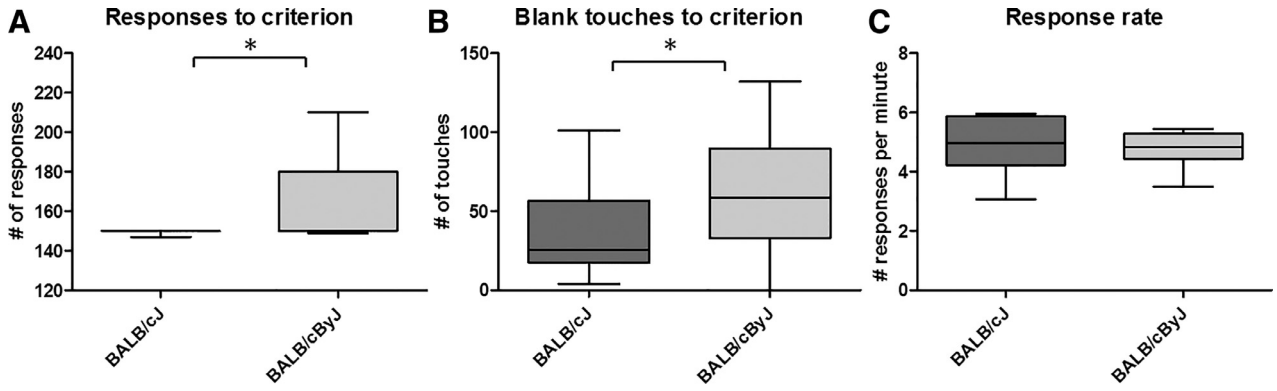

Fig. 2 Acquisition phase of Appetitive Extinction learning comparing BALB/cJ to BALB/cByJ mice. (A) The total number of responses to reach the criterion of having 30 responses to the stimulus within 12.5 min for 5 consecutive days. (B) The total number of touches on screen until reaching criterion where no stimulus was present. (C) The mean response rate measured as the number of response per minute until reaching criterion. Data are presented as medians with corresponding IQR; $^{*}p < 0.05$.

Animals that died during the experiment and outliers (±1.5* Interquartile range) were removed before performing the statistical analysis and FDR correction was applied for multiple comparisons. All analyses were done using SPSS (IBM, version 22.0). All boxplots were created using interquartile range (IQR) and both the boxplots and line graphs were visualised with GraphPad Prism (version 5.03).

3. Results

Here we report whether cognitive flexibility is altered in the acquisition and extinction of a visual appetitive extinction paradigm in BALB/cJ and BALB/cByJ mice. We further assess if the ability to learn influences underlying impulse control and appetitive extinction in these sub-strains.

3.1. Acquisition: sub-strain performance comparison

The data of the acquisition phase of the touchscreen task can be found in **Supplementary Table 1**, starting with our primary measures: number of sessions (containing maximum 30 trials each) and trials required to reach criterion. BALB/cJ mice showed a significantly lower total number of sessions ($U = 49.5$; $P = 0.020$; $N = 26$) as well as total number of responses (trials) to reach the set criterion ($U = 50$; $P = 0.039$; $N = 26$; Fig. 2(A)). In addition, the BALB/cJ mice had a lower number of blank touches compared to the BALB/cByJ to reach the 5-day criterion ($U = 42.5$; $P = 0.033$; $N = 26$; Fig. 2(B)), while the mean response rate between

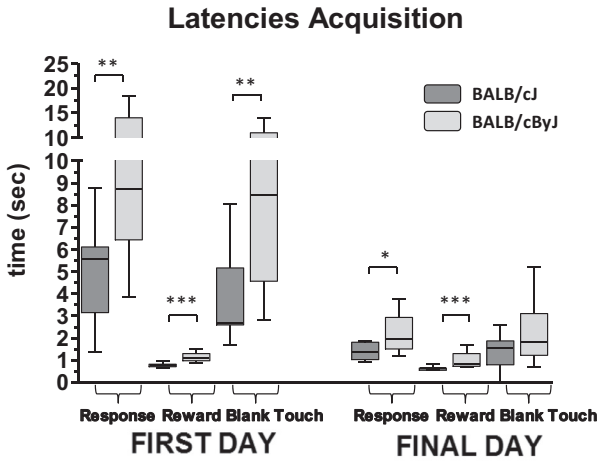

Fig. 3 Latencies during acquisition phase of Appetitive Extinction learning comparing BALB/cJ to BALB/cByJ mice. For both the first and final day of acquisition when criterion was reached it shows: (1) The latency to correctly touch the stimulus on the first and final day. (2) The latency to retrieve the reward after a correct touch response was made. (3) The latency for blank touches. Data are presented as medians with corresponding IQR; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

the BALB/c sub-strains was not different ($t_{23} = -0.46$; $P = 0.647$; Fig. 2(C)). The lower number of sessions, responses to reach criterion and blank touches indicates that the BALB/cJ mice learn the task faster and make less errors. Both sub-strains are able to acquire the task equally well (as seen by the fact that the response rate is similar).

Mean latencies (response, reward and blank touch) were examined as measures of reward sensitivity and motivation in the acquisition phase (**Supplementary Fig. 2**). For all three latency measures, the BALB/cJ mice showed lower values compared to BALB/cByJ mice, meaning that they touched faster (response latency: $U = 29$; $P = 0.008$; $N = 25$), pick up the reward faster (reward latency $U = 9$; $P < 0.001$, $N = 24$) and touch the screen faster on the locations where no stimulus is presented (blank touch latency $U = 36$; $P = 0.025$, $N = 25$). These differences were also observed at day 1 (response latency: $t_{20.36} = 3.738$; FDR adjusted $P = 0.003$, reward latency: $U = 4$; FDR adjusted $P < 0.001$; $N = 25$, and blank touch latency: $U = 15$; FDR adjusted $P = 0.004$; $N = 23$) and on the final day of the acquisition (response latency: $U = 28$; FDR adjusted $P = 0.022$; $N = 23$, reward latency: $U = 5$; FDR adjusted $P < 0.001$; $N = 25$ and blank touch latency: $U = 46.5$; FDR adjusted $P = 0.094$; $N = 25$; Fig. 3).

The comparison between the first and final day of acquisition showed that the BALB/c sub-strains had significant shorter latencies ((response latency: $Z_{22} = -4.107$; $P < 0.001$; reward latency: $Z_{23} = -3.559$; $P < 0.001$; blank touch latency: $Z_{21} = -3.875$; $P < 0.001$ (Fig. 3)) by the final day compared to first.

3.2. Extinction: sub-strain performance comparison

During the extinction phase, both of the BALB/c sub-strains tested inhibited their responses and had more omissions over time. This was observed in general over the course of the 15 sessions accompanied by increases in the number of blank touches per session (**Supplementary Fig. 3(A) and (B) and Supplementary Table 2**). For the primary and additional measures, the data shows that the number of sessions ($U = 86$; $P = 0.836$; $N = 27$), responses ($t_{24} = -0.497$; $P = 0.624$; Fig. 4(A)), blank touches ($t_{25} = 0.508$; $P = 0.616$; Fig. 4(B)) and the response rate ($U = 77$; $P = 0.719$; $N = 26$; Fig. 4(C)) to reach criterion were not significantly different between the BALB/cJs and BALB/cByJs. These results indicate that both sub-strains are able to inhibit the response to the stimulus equally when adjusting for the number of sessions each animal successfully had.

Similar to the acquisition phase, the mean latencies were analysed as measures of reward sensitivity and motivation for the extinction phase (Fig. 5). The reward latency showed a significant decrease in the BALB/cJ compared to the BALB/cByJ ($t_{25} = 2.965$; $P = 0.007$). No differences were found in the other two latency measures (response latency: $t_{25} = 0.995$; $P = 0.329$ and blank touch latency $t_{25} = 1.231$; $P = 0.230$). The latencies on the first day of the extinction phase showed that only the response latency was different between the sub-strains (response latency: $U = 19$; FDR adjusted $P = 0.002$; $N = 26$; reward latency: $t_{25} = 0.177$; FDR adjusted $P = 0.861$; blank touch latency; $t_{24} = -0.005$; FDR adjusted $P = 0.996$, Fig. 6). For the latencies on the final day (Fig. 6), the reward latency showed a significant decrease ($U = 38$; FDR adjusted $P = 0.022$; $N = 27$), while the response- and blank touch latency did not differ ($U = 81$; FDR adjusted $P = 0.877$; $N = 26$, $t_{25} = 1.343$; FDR adjusted $P = 0.382$, respectively).

The latencies of the first and last day of the extinction were compared to see if changes in the extinction phase were observed over time. All latencies increased during the response inhibition process during extinction (response latency: $Z = -2.704$; $P = 0.007$; $N = 25$; reward latency: $Z = -4.300$; $P < 0.001$; $N = 27$; blank touch latency: $Z = -3.365$; $P = 0.001$; $N = 26$).

3.3. Extinction:sub-strain performance comparison based on reaching criterion

During the extinction phase only half of the animals reached the criterion. We investigated the performance of the animals that reached criterion with the ones that did not reach it using the data until reaching criteria (for the reaching group) or of all 15 sessions per animal (for the non-reaching group). The results of this analysis can be found in **Supplementary Table 3**. Briefly, we did not find differences between the sub-strains for the reaching group nor for the not reaching group (Fig. 7**)**.

Within the reaching group the reward latency was lower in the BALB/cJ mice compared to the BALB/cByJ mice ($t_{12} = -2.826$; $P = 0.015$), while the response latency was decreased in the not reaching BALB/cJ sub-strain ($t_{11} = -2.880$; $P = 0.015$). The other latencies did not show any differences (reached: response $t_{12} = 0.499$; $P = 0.627$, blank touch $t_{12} = -0.957$; $P = 0.358$, not reached: reward $t_{11} = -1.387$; $P = 0.193$; blank touch $t_{11} = -0.704$; $P = 0.496$) (**Supplementary Fig. 4**). The comparison between day 1 and either

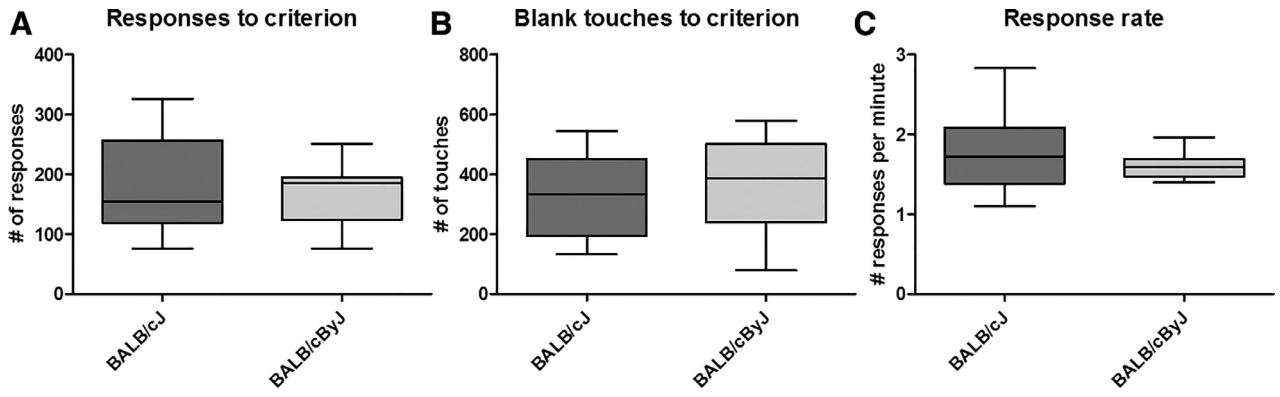

Fig. 4 Ability to 'unlearn' during extinction phase of Appetitive Extinction learning comparing BALB/cJ to BALB/cByJ mice. (A) The number of responses made to reach criterion. (B) The number of touches on screen where no stimulus was present. (C) The mean response rate measured as the number of response per minute until reaching criterion. Data are presented as medians with corresponding IQR.

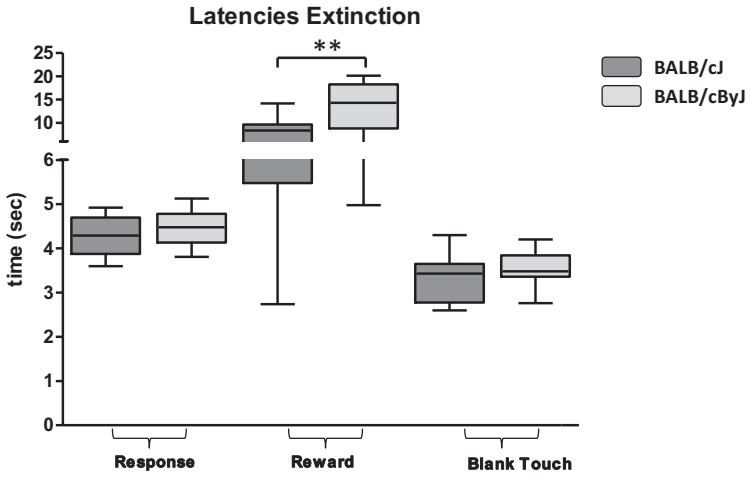

Fig. 5 Comparison of mean latencies of BALB/cJ and BALB/cByJ mice during extinction until reaching criterion with a maximum of 15 sessions. From left to right: (1) The mean latency to respond to stimulus. (2) The latency to check the tray for reward after responding. (3) The latency for blank touches. Data are presented as medians with corresponding IQR; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p \leq 0.001$.

the reaching day or day 15 can be found in **Supplementary Fig. 5 and Supplementary Results**.

4. Discussion

The current study investigated the ability to stop responding to a previously reinforced stimulus in BALB/cJ and BALB/cByJ mice sub-strains during an appetitive extinction paradigm, in order to examine the learning (acquisition) of new rewarded behaviour and the ability to inhibit it (extinction) when the stimulus-reward association is broken.

Our results demonstrate that both sub-strains are able to acquire the task, though with possible different learning strategies. The speed of acquisition is faster in BALB/cJ mice compared to BALB/cByJ mice, without a reduction in response rate to reach criterion. In addition, the latency to respond to the stimulus, collect the reward and touch the screen on locations where no stimulus was presented, was shorter in the BALB/cJ sub-strain during acquisition overall and measures on the first and final day. This confirms and extends our data that BALB/cJ mice are more sensitive to rewards than BALB/cByJ mice, not only in reversal learning (Jager, 2019), but also during acquisition of appetitive extinction learning. The number of blank touches was also lower in BALB/cJ compared to BALB/cByJ mice in general (including reaching the criterion). This could be interpreted as a reduced number of mistakes made by the BALB/cJ mice (compared to BALB/cByJ sub-strains). Together with the shorter latencies, the source of the decreased number of blank touches may indicate improved error detection, increased cue sensitivity or salience to the rewarded cue, or decreased impulsivity in BALB/cJ mice when acquiring the task. The BALB/cByJ sub-strain is, on the other hand, more perseverative as they have more blank touches throughout the acquisition phase, i.e. they touch the screen more often where no stimulus is presented.

The BALB/cJ and BALB/cByJ sub-strains showed no difference during the extinction phase: the performance in response inhibition was equal among all animals. Both strains

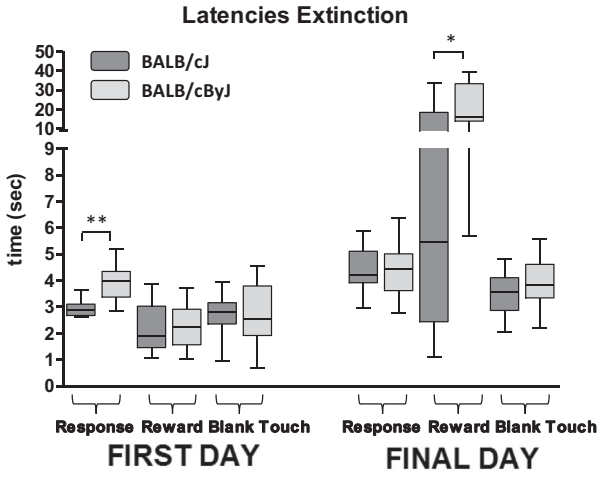

Fig. 6 Latencies during extinction phase of Appetitive Extinction learning comparing BALB/cJ to BALB/cByJ. For both the first and final day of extinction (day 15) it shows: (1) The latency to touch the stimulus on the first and final day. (2) The latency to check for a reward. (3) The latency for blank touches. Data are presented as medians with corresponding IQR; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

increased the number of omissions and blank touches, and performed with higher latencies over the course of the 15 sessions of extinction. The same holds for the performance until criterion in the primary and additional measures. However, the BALB/cJ animals were faster when checking for a reward (overall and in the last session), which was observed in the acquisition phase as well. Therefore, it seems that these animals are more reward driven even though no rewards are provided at all during the extinction phase, meaning this reward sensitivity of BALB/cJ mice persists over time and in the absence of reward. Whether this is independent of the reward, reward type or magnitude will require additional studies. Interestingly, on the first day the BALB/cJ mice responded faster to the stimulus, though this difference disappeared over time. Therefore, the difference in response latency on day one is likely to be caused by the performance during the acquisition, as a lower response latency was present in BALB/cJ mice throughout the acquisition phase and disappeared as inhibition occurred during the extinction phase.

Within the 15 sessions of the extinction phase, approximately (only) half of the animals per sub-strain reached the criterion. Separating each sub-strain in groups based on their performance showed that the reward latency difference observed in the extinction phase was due to the mice reaching criterion (reached <75% omissions for two consecutive days): the BALB/cJ mice checked for a reward faster than the BALB/cByJ mice that reached criterion. On the other hand, in the group that did not reach criterion, the BALB/cJ mice had a shorter session compared to the BALB/cByJ due to significant faster responses on the first day and throughout the extinction phase overall (**Supplementary Table 3**). Taking these results together, it seems that there are subgroups within the BALB/cJ mice: BALB/cJ mice that reached criterion are more reward driven, whereas the BALB/cJ mice that did not reach the criterion, make more mistakes during extinction compared to BALB/cByJ. Whether this represents an improved ability to stop responding to previously acquired stimulus-reward associations within the BALB/cJ mice or the adoption of a different behavioural strategy compared to the BALB/cByJ mice remains to be clarified.

We have also retrospectively tested whether those reaching the extinction criterion were different not only during extinction but also during acquisition in the existing BALB/cJ and BALB/cByJ extinction performance groups (Supplementary Table 4; Supplementary Figs. 6 and 7). In the between strain (BALB/cJ vs. BALB/cByJ mice) comparison, BALB/cJ mice not reaching the extinction criterion, were fast learners, made less errors (or made those errors faster on the first day) and respond faster (both on the first day and subsequently) when compared to matching BALB/cByJ datasets. In both those reaching and not reaching the extinction criterion, BALB/cJ mice were clearly more reward sensitive independent of their ability to inhibit the learnt strategy in the extinction phase when comparing them to the BALB/cByJ mice. BALB/cJ mice respond faster to not only reward but also to blank touches. This leads to the question: is this decrease in latency to respond related to adopting a more 'automatic' strategy where they do not

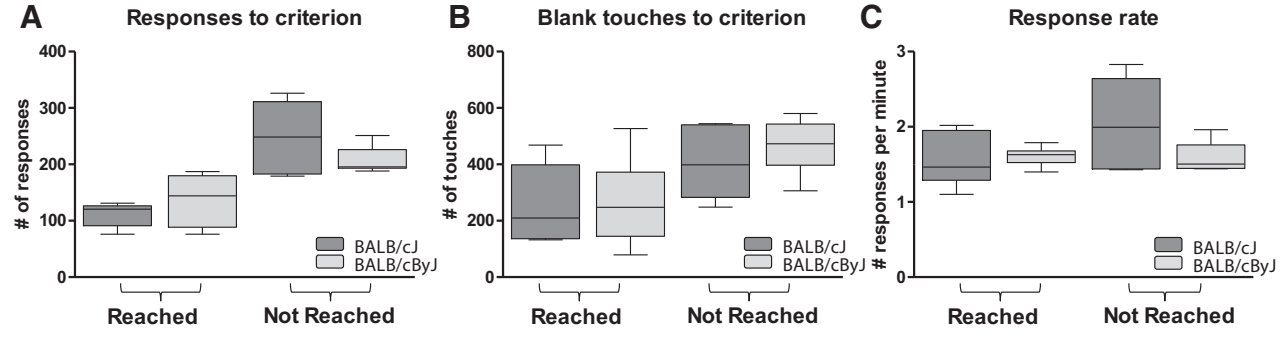

Fig. 7 Comparison of BALB/cJ and BALB/cByJ mice that reached or not reached the extinction criterion during extinction phase of Appetitive Extinction learning. (A) The number of responses made to reach criterion for reaching and not reaching group. (B) The number of touches on screen were no stimulus was present for the reaching and not reaching group. (C) The mean response rate measured as the number of response per minute until reaching criterion for the reaching and not reaching group. Data are presented as medians with corresponding IQR.

carefully consider their response? It would be interesting to compare the late phase of reversal learning (which may reflect habitual responding) between BALB/cJ and BALB/cByJ to evaluate this further. Taken together, it appears that the non-reaching BALB/c mice drive the observed effects in the analysis of acquisition data without sub-groups.

In the within strain comparison of the same data, we also compared the acquisition data of those BALB/cJ mice reaching and not reaching the learning criterion at the extinction phase. There is a decrease in time to respond (reduced mean response and reward latencies) in those BALB/cJ mice not reaching the criterion, which may suggest that they don't have the ability to inhibit their response due to the (overly) rapid responses. In contrast, the BALB/cByJ mice that do not reach the extinction criterion learn slower (as measured by the increase of the number of sessions and trials needed to reach criterion and the increased amount of time required per session), i.e. they can learn the strategy but are slower to learn this but also retain the ability to inhibit these responses. Whether the speed of information acquisition affects the quality of learning would be worth investigating further. Examining this in a longer protocol design with more sessions might provide the opportunity for these BALB/cByJ mice to learn to inhibit these responses.

Our data demonstrate that there are distinctions in learning and extinction strategies of the BALB/c sub-strains. Our results on extinction add to previous reports on positively reinforced reversal learning and demonstrate the presence of behavioural subgroups within the BALB/cJ strain based on their performance (Graybeal, 2014). Many studies to date have reported differential effects in terms of persistent responding after changes in rules (perseveration) between animals tested in both reversal learning and extinction (Annett et al., 1989; Brigman, 2008; Castane et al., 2010; Izquierdo and Murray, 2005; Karlsson, 2009), which is confirmed by our extinction results. Whether this suggests that these tasks measure different kinds of cognitive flexibility, which is differentially regulated by other neural / molecular substrates, has been investigated by others but not completely elucidated (Clarke et al., 2008; Hamilton and Brigman, 2015; Izquierdo and Jentsch, 2012). A possible explanation for the difference between the performance during the tasks can be that both implement different dynamic adjustments in the occurrence of reward. Both tasks involve metrics related to response inhibition or inhibitory control: during reversal learning, two stimuli are involved and subjects must suppress one response while engaging actively in another to obtain reward. As a consequence, there remains a strong motivational impulse to respond post reversal (selective response inhibition or behavioural switching). In extinction, however, the subject may simply inhibit the conditional response to a single stimulus, reflecting the importance of conserving energy when actions no longer result in reward (general behavioural inhibition) (Clarke et al., 2008; Izquierdo and Jentsch, 2012).

Deficits with inhibition during an extinction paradigm have been reported in clinical ADHD and ASD cases (Murphy 2002; Sagvolden, 2005; Schmitt, 2018), aspects of which are modelled by BALB/c sub-strains. Our earlier studies suggest that BALB/cJ and BALB/cByJ mice perform differently in reversal learning (Jager, 2019), and fear extinction (Sanford et al., 2003; Schimanski and Nguyen, 2005). More specifically, our previous observations were that the BALB/cJ mice (in contrast to the BALB/cByJ), show perseveration in reversal learning associated with punishment (Jager, 2019) and this is extended to the appetitive extinction in this study. Furthermore, results from us and others also show that the BALB/cJ, but not BALB/cByJ mice, demonstrate reduced social interaction (Brodkin, 2007; Fairless, 2013; van Heukelum, 2019). In the context of our current findings, it may suggest that BALB/cJ mice are more sensitive to their environment and changes within it. Together with other unpublished data sets in our laboratory (social conditioned place preference and three chamber task) and other research, this suggest that the BALB/cJ mouse model demonstrates some features relevant to autism-related traits (Velez, 2010).

In the current study, data regarding reward latency in the acquisition and extinction phase was utilised as a metric both of impulsivity and reward sensitivity. This metric is differentially interpreted by a number of researchers as indicating (i) the willingness to access the reward and therefore in part reflects how motivated the animal is for the reward, (ii) the sensitivity of the animal for the reward cue and (iii) an index of activation. A more thorough dissection of impulsivity, motivation and reward sensitivity in these strains would be useful. To this end, we have already tested BALB/cJ and BALB/cByJ mice in the 5-choice serial reaction time task. No strain-difference in premature responses (an index of impulsivity) is observed, though decreased reward latencies in BALB/cJ mice persist across tasks. Assessment of motivation would therefore be a useful next step e.g. in the progressive ratio schedule task linked to reward. Additionally, while the underlying molecular substrates of the difference in extinction learning between the BALB/c sub-strains is unknown, it may be useful to investigate the genetic differences between them. The breeding of the BALB/c strain into two separate sub-strains (BALB/cJ and BALB/cByJ), may have introduced errors by producing new alleles and/or spontaneous mutations. This may underlie the phenotypic differences of BALB/c sub-strains that are relevant to ASD and ADHD (Velez, 2010). As such, next generation sequencing of the BALB/cJ and BALB/cByJ sub-strains (but not those tested in operant chambers) has now been performed in collaboration with others, to identify genetic substrates which underlie the behavioural differences between BALB/c sub-strains.

In conclusion, we demonstrate that both BALB/cJ and BALB/cByJ mice are able to learn the association between stimulus and response in the acquisition of the appetitive extinction task. The strategy used to learn the association by each of the BALB/c sub-strains may differ, as the BALB/cJ mice acquire this task more rapidly than BALB/cByJ mice and seem to be more reward driven. The ability to extinguish a learnt response was comparable between the sub-strains, but there are variations in their ability to do this within the BALB/cJ sub-strain. BALB/cJ animals that reached the criterion were more reward driven. In contrast, BALB/cJ mice failing to reach the set criterion during extinction show more erroneous behaviour compared to those BALB/cByJ mice that also fail to reach criterion. Additionally, the animals that did not reach the criterion in the extinction phase, are driving the changes observed during acquisition. Taken together, we demonstrate that the ability

to learn, as defined by reaching criterion, influences extinction performance and cognitive flexibility in BALB/c mice.

Conflict of interest

Jan Buitelaar was a consultant to/member of advisory board of/and/or speaker for Janssen-Cilag BV, Eli Lilly, Shire, Novartis, Roche and Servier. Jeffrey Glennon has in the past three years been a consultant to Boehringer Ingelheim GmbH. Neither Jan Buitelaar nor Jeffrey Glennon are employees of any of these companies, and neither are stock shareholders of any of these companies. Neither the funding organizations nor any of the industrial consultancies listed has had any involvement with the conception, design, data analysis and interpretation, review and/or any other aspects relating to this paper. All other authors have no conflicts of interest to declare.

Contributors

Authors SAD, AJ, CAO, JKB and JCG were involved in the design of the study. Authors SAD and AJ performed the behavioural tests. Author SAD and AAV undertook the statistical analysis, and author SAD together with author JCG were involved in making the graphical representation of the data. The draft manuscript was written by SAD, AAV and JCG. All authors contributed to and have approved the final manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2019.02.007.

References

Annett, L.E., McGregor, A., Robbins, T.W., 1989. The effects of ibotenic acid lesions of the nucleus accumbens on spatial learning and extinction in the rat. Behav. Brain Res. 31 (3), 231–242.

Argyropoulos, A., Gilby, K.L., Hill-Yardin, E.L., 2013. Studying autism in rodent models: reconciling endophenotypes with comorbidities. Front. Hum. Neurosci. 7, 417.

Bari, A., Robbins, T.W., 2013. Inhibition and impulsivity: behavioral and neural basis of response control. Prog. Neurobiol. 108, 44–79.

Berlin, G.S., Hollander, E., 2014. Compulsivity, impulsivity, and the DSM-5 process. CNS Spectr. 19 (1), 62–68.

Bouton, M.E., 2004. Context and behavioral processes in extinction. Learn. Mem. 11 (5), 485–494.

Brigman, J.L., et al., 2008. Impaired discrimination learning in mice lacking the NMDA receptor NR2A subunit. Learn. Mem. 15 (2), 50–54.

Brodkin, E.S., 2007. BALB/c mice: low sociability and other phenotypes that may be relevant to autism. Behav. Brain Res. 176 (1), 53–65 (0166-4328 (Print)).

Brodkin, E.S., et al., 2004. Social approach-avoidance behavior of inbred mouse strains towards DBA/2 mice. Brain Res. 1002 (1-2), 151–157.

Burket, J.A., et al., 2016. Characterization of gait and olfactory behaviors in the Balb/c mouse model of autism spectrum disorders. Brain Res. Bull. 122, 29–34.

Bussey, T.J., et al., 2012. New translational assays for preclinical modelling of cognition in schizophrenia: the touchscreen testing method for mice and rats. Neuropharmacology 62 (3), 1191–1203.

Castane, A., Theobald, D.E., Robbins, T.W., 2010. Selective lesions of the dorsomedial striatum impair serial spatial reversal learning in rats. Behav. Brain Res. 210 (1), 74–83.

Clarke, H.F., Robbins, T.W., Roberts, A.C., 2008. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. J. Neurosci. 28 (43), 10972–10982.

Cooper, J.O., Heron, T.E., Heward, W.L., 2007. 'Chapter 21: Extinction', *Applied Behaviour Analysis*. New Jersey, Pearson Education.

Craig, F., et al., 2016. A review of executive function deficits in autism spectrum disorder and attention-deficit/hyperactivity disorder. Neuropsychiatr. Dis. Treat. 12, 1191–1202.

Dow, H.C., Kreibich, A.S., Kaercher, K.A., Sankoorikal, G.M., Pauley, E.D., Lohoff, F.W., Ferraro, T.N., Li, H., Brodkin, E.S., 2011. Genetic dissection of intermale aggressive behavior in BALB/cJ and A/J mice. Genes Brain Behav 10 (1), 57–68. doi:10.1111/j.1601-183X.2010.00640.x, Epub 2010 Oct 5.

Fairless, A.H., et al., 2013. Development of home cage social behaviors in BALB/cJ vs. C57BL/6J mice. Behav. Brain Res. 237, 338–347.

Fairless, A.H., et al., 2008. Low sociability is associated with reduced size of the corpus callosum in the BALB/cJ inbred mouse strain. Brain Res. 1230, 211–217.

Fineberg, N.A., et al., 2010. Probing compulsive and impulsive behaviors, from animal models to endophenotypes: a narrative review. Neuropsychopharmacology 35 (3), 591–604.

Graybeal, C., et al., 2014. Strains and stressors: an analysis of touchscreen learning in genetically diverse mouse strains. PLoS One 9 (2), e87745.

Hamilton, D.A., Brigman, J.L., 2015. Behavioral flexibility in rats and mice: contributions of distinct frontocortical regions. Genes Brain Behav. 14 (1), 4–21.

Horner, A.E., et al., 2013. The touchscreen operant platform for testing learning and memory in rats and mice. Nat. Protoc. 8 (10), 1961–1984.

Izquierdo, A., Murray, E.A., 2005. Opposing effects of amygdala and orbital prefrontal cortex lesions on the extinction of instrumental responding in macaque monkeys. Eur. J. Neurosci. 22 (9), 2341–2346.

Izquierdo, A., Jentsch, J.D., 2012. Reversal learning as a measure of impulsive and compulsive behavior in addictions. Psychopharmacology (Berl) 219 (2), 607–620.

Jacome, L.F., et al., 2011. Genetically inbred Balb/c mice differ from outbred Swiss webster mice on discrete measures of sociability: relevance to a genetic mouse model of autism spectrum disorders. Autism Res. 4 (6), 393–400.

Jager, A., Dam, S.A., et al. (Submitted for publication), 2019 'Modulation of Cognitive Flexibility by Reward and Punishment in BALB/cJ and BALB/cByJ mice'.

Jager, A., et al., 2017. Cortical control of aggression: GABA signalling in the anterior cingulate cortex. Eur. Neuropsychopharmacol. pii: S0924-977X(17)32033-3. doi: 10.1016/j.euroneuro.2017.12.007. [Epub ahead of print].

Karlsson, R.M., et al., 2009. Assessment of glutamate transporter GLAST (EAAT1)-deficient mice for phenotypes relevant to the negative and executive/cognitive symptoms of schizophrenia. Neuropsychopharmacology 34 (6), 1578–1589.

Lamanna, A.L., et al., 2017. Risk factors for the existence of attention deficit hyperactivity disorder symptoms in children with autism spectrum disorders. Neuropsychiatr. Dis. Treat. 13, 1559–1567.

Lattal, K.M., Lattal, K.A., 2012. Facets of Pavlovian and operant extinction. Behav. Process. 90 (1), 1–8.

Laviola, G., et al., 2017. Low empathy-like behaviour in male mice associates with impaired sociability, emotional memory, physiological stress reactivity and variations in neurobiological regulations. PLoS One 12 (12), e0188907.

Mar, A.C., et al., 2013. The touchscreen operant platform for assessing executive function in rats and mice. Nat. Protoc. 8 (10), 1985–2005.

Martin, J., et al., 2014. Biological overlap of attention-deficit/hyperactivity disorder and autism spectrum disorder: evidence from copy number variants. J. Am. Acad. Child Adolesc. Psychiatry 53 (7), 761 70e26.

Miltenberger, R.G., 2008. 'Chapter 5: Extinction', *Behavior Modification*. Wadsworth Publishing, Belmont, CA.

Miyake, A., Friedman, N.P., 2012. The nature and organization of individual differences in executive functions: four general conclusions. Curr. Dir. Psychol. Sci. 21 (1), 8–14.

Moy, S.S., et al., 2007. Mouse behavioral tasks relevant to autism: phenotypes of 10 inbred strains. Behav. Brain Res. 176 (1), 4–20.

Murphy, P., 2002. Inhibitory control in adults with attention-deficit/hyperactivity disorder. J. Atten. Disord. 6 (1), 1–4.

Musser, E.D., et al., 2014. Shared familial transmission of autism spectrum and attention-deficit/hyperactivity disorders. J. Child Psychol. Psychiatry 55 (7), 819–827.

Naaijen, J., et al., 2015. Fronto-striatal glutamatergic compounds in compulsive and impulsive syndromes: a review of magnetic resonance spectroscopy studies. Neurosci. Biobehav. Rev. 52, 74–88.

Oomen, C.A., et al., 2013. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. Nat. Protoc. 8 (10), 2006–2021.

Pinto, R., et al., 2016. The genetic overlap of attention-deficit/hyperactivity disorder and autistic-like traits: an investigation of individual symptom scales and cognitive markers. J. Abnorm. Child Psychol. 44 (2), 335–345.

Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33 (1), 56–72.

Robbins, T.W., et al., 2012. Neurocognitive endophenotypes of impulsivity and compulsivity: towards dimensional psychiatry. Trends Cogn. Sci. 16 (1), 81–91.

Rommelse, N.N., et al., 2010. Shared heritability of attention-deficit/hyperactivity disorder and autism spectrum disorder. Eur. Child Adolesc. Psychiatry 19 (3), 281–295.

Rommelse, N.N., et al., 2011. A review on cognitive and brain endophenotypes that may be common in autism spectrum disorder and attention-deficit/hyperactivity disorder and facilitate the search for pleiotropic genes. Neurosci. Biobehav. Rev. 35 (6), 1363–1396.

Sagvolden, T., et al., 2005. A dynamic developmental theory of attention-deficit/hyperactivity disorder (ADHD) predominantly hyperactive/impulsive and combined subtypes. Behav. Brain Sci. 28 (3), 397–419 discussion 19-68.

Sanford, L.D., Fang, J., Tang, X., 2003. Sleep after differing amounts of conditioned fear training in BALB/cJ mice. Behav. Brain Res. 147 (1-2), 193–202.

Sankoorikal, G.M., et al., 2006. A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. Biol. Psychiatry 59 (5), 415–423.

Schimanski, L.A., Nguyen, P.V., 2005. Mouse models of impaired fear memory exhibit deficits in amygdalar LTP. Hippocampus 15 (4), 502–517.

Schmitt, L.M., et al., 2018. Cognitive mechanisms of inhibitory control deficits in autism spectrum disorder. J. Child Psychol. Psychiatry 59 (5), 586–595. doi:10.1111/jcpp.12837, Epub 2017 Oct 20.

Talpos, J.C., et al., 2009. A novel touchscreen-automated paired-associate learning (PAL) task sensitive to pharmacological manipulation of the hippocampus: a translational rodent model of cognitive impairments in neurodegenerative disease. Psychopharmacology (Berl) 205 (1), 157–168.

Tran, N.Q.V., Miyake, K., 2017. Neurodevelopmental disorders and environmental toxicants: epigenetics as an underlying mechanism. Int. J. Genom. 2017, 7526592.

van Heukelum, S., et al. 2019 (Submitted for publication),'Somatostatin- and Parvalbumin-Expressing Interneurons in Anterior Cingulate and Midcingulate Cortex Fulfil Opposite Roles in Aggression and Social Withdrawal in The BALB/cJ mouse'.

Velez, L., et al., 2010. Differences in aggressive behavior and DNA copy number variants between BALB/cJ and BALB/cByJ substrains. Behav. Genet. 40 (2), 201–210.

Zarcone, T.J., Chen, R., Fowler, S.C., 2004. Differential acquisition of food-reinforced disk pressing by CD-1, BALB/cJ and C57BL/6J mice. Behav. Brain Res. 152 (1), 1–9.