Drug and Alcohol Dependence

Impact of adolescent methamphetamine use on social cognition A human-mice reverse translation study

--Manuscript Draft--

Manuscript Number:	LP-21-1262R1
Article Type:	Full Length Article
Keywords:	Methamphetamine; Age of onset; Adolescence; Social Cognition; Social interaction; Dopamine
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Abstract:	Background Methamphetamine dependence is associated with social cognition deficits that may underpin negative social outcomes. However, there are considerable inter-individual differences in social cognition within people with methamphetamine dependence, with age of onset of methamphetamine use being a potential contributing factor. Materials and methods We conducted two sequential studies examining the link between age of onset of methamphetamine use (adolescence versus young adulthood) and performance in social cognition tests: (1) a human cross-sectional study in 95 participants with methamphetamine dependence varying in age of onset (38 with adolescent onset and 57 with adult onset) and 49 drug-naïve controls; (2) a mice study in which we tested the effects of methamphetamine exposure during adolescence versus young adulthood on social interaction and aggression, and their potential neurochemical substrates in the striatal dopaminergic system. Results We initially showed that people with methamphetamine dependence who started use in adolescence had higher antisocial beliefs (p=0.046, Cohen's d=0.42) and worse emotion recognition (p=0.031, Cohen's d=0.44) than those who started use during adulthood. We reasoned that this could be due to either social cognition deficits leading to earlier onset of methamphetamine use, or methamphetamine-induced neuroadaptive effects specific to adolescence. Mice experiments showed that methamphetamine exposure during adolescence specifically decreased social investigation during social interaction and upregulated striatal tyrosine hydroxylase (p<0.05, Bonferroni corrected). There was no evidence of adolescent-specific methamphetamine effects on aggression or other measures of dopaminergic function.Conclusion

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Word count: 6062

Impact of adolescent methamphetamine use on social cognition: A human-mice reverse translation study

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Abstract

Background: Methamphetamine dependence is associated with social cognition deficits that may underpin negative social outcomes. However, there are considerable inter-individual differences in social cognition within people with methamphetamine dependence, with age of onset of methamphetamine use being a potential contributing factor.

Materials and methods: We conducted two sequential studies examining the link between age of onset of methamphetamine use (adolescence versus young adulthood) and performance in social cognition tests: (1) a human cross-sectional study in 95 participants with methamphetamine dependence varying in age of onset (38 with adolescent onset and 57 with adult onset) and 49 drug-naïve controls; (2) a mice study in which we tested the effects of methamphetamine exposure during adolescence versus young adulthood on social interaction and aggression, and their potential neurochemical substrates in the striatal dopaminergic system.

Results: We initially showed that people with methamphetamine dependence who started use in adolescence had higher antisocial beliefs ($p=0.046$, Cohen's $d=0.42$) and worse emotion recognition ($p=0.031$, Cohen's $d=0.44$) than those who started use during adulthood. We reasoned that this could be due to either social cognition deficits leading to earlier onset of methamphetamine use, or methamphetamine-induced neuroadaptive effects specific to adolescence. Mice experiments showed that methamphetamine exposure during adolescence specifically decreased social investigation during social interaction and upregulated striatal tyrosine hydroxylase ($p<0.05$, Bonferroni corrected). There was no evidence of adolescent-specific methamphetamine effects on aggression or other measures of dopaminergic function.

Conclusion: Together, translational findings demonstrate heightened sensitivity to methamphetamine effects on social cognition during adolescence.

Keywords: Methamphetamine; Age of onset; Adolescence; Social Cognition; Social interaction; Dopamine

1. Introduction

Methamphetamine dependence has been consistently associated with social cognition deficits of large effect size, which bear clinical significance (Kim et al., 2011; Potvin et al., 2018). These deficits may have a significant impact on real-world social behaviours, such as aggression and social exclusion, which contribute to the burden of disease attributable to methamphetamine dependence (Homer et al., 2008; Tae et al., 2011). However, there are significant inter-individual differences in social cognition within people with methamphetamine dependence, and the mechanisms underlying such differences remain unclear (Payer et al., 2012; Uhlmann et al., 2018).

Methamphetamine use during adolescence has been associated with neuroadaptations affecting brain structure and metabolism and alterations in higher-order cognitive functions, suggesting that age of onset may be a key contributor to inter-individual differences in social cognition (Kim et al., 2018; Lyoo et al., 2015). In support of this assumption, rodent models have shown that chronic regimens of methamphetamine administration during adolescence impair social behaviour (Manning and van den Buuse, 2016). These models have also been instrumental in demonstrating the direct neuroadaptive effects of methamphetamine use on striatal dopamine systems, which are implicated in the development of social cognition (Kopec et al., 2019). Furthermore, in humans, adolescent brain maturation overlaps with consolidation of personality (Vijayakumar et al., 2014). Thus, in addition to drug-related effects, developmental variations in personality function can affect social cognition (Churchwell et al., 2012). Specifically, dysfunctional beliefs that feature prominently in antisocial and other adolescent-onset personality disorders are significantly associated with the emotion recognition deficits observed in people with methamphetamine dependence (Hanegraaf et al., 2020). Methamphetamine dependence seems to be specifically associated with impaired recognition of anger, which has been independently linked with hostility and

aggression biases and brain lesions in the striatum (Calder et al., 2004; Davies et al., 2018). Altogether, both adolescent-specific personality features and drug-related effects on the maturing adolescent brain may underpin the relationship between age of onset of methamphetamine use and social cognition deficits.

Here, we report findings from two sequential studies examining the link between age of onset of methamphetamine use (adolescence versus adulthood) and social cognition: (1) a human cross-sectional study among people with methamphetamine dependence varying in age of onset of methamphetamine use; (2) a mice experiment in which we tested the differential impact of methamphetamine administration during adolescence versus young adulthood on social cognition, as well as its putative striatal dopaminergic mechanisms (Liu et al., 2017). The two studies were designed to provide complementary insights: the human study sought to establish the link between age of onset and social cognition deficits, while controlling for severity and duration of methamphetamine use. However, this study could not ascertain if premorbid social cognition deficits led to early use of methamphetamine (via negative reinforcement or coping mechanisms), or if, alternatively, early use of methamphetamine led to social cognition deficits (via drug-related neuroadaptations). The mice study was incorporated to determine if methamphetamine use during adolescence (versus young adulthood) directly disrupts social cognition and their neural underpinnings. Although rodent paradigms for social cognition measurement have inherent limitations concerning validity, we used a well-validated social interaction paradigm that has been proposed as one of the most suitable for translational studies (Millan and Bales, 2013). In addition, we used a rodent aggression paradigm given the previously proposed links between social cognition and aggression in methamphetamine dependence (Homer et al., 2008; Kim et al., 2011). With regards to neural underpinnings, we focused on the striatum to maximise translational similarity, as human evidence has shown that striatum lesions lead to social

cognition alterations (Calder et al., 2004); that is the same directionality that we sought to examine in our mice study (i.e. adolescent methamphetamine use leading to social cognition deficits). An additional advantage of the striatum, relative to prefrontal and anterior cingulate cortex regions previously associated with social cognition deficits in human methamphetamine dependence (Kim et al., 2008; Payer et al., 2011), is a clear-cut homology in this region between humans and mice (Balleine and O'Doherty, 2010), which is more difficult to establish in prefrontal / anterior cingulate cortex regions (Carlen, 2017; van Heukelum et al., 2020). We initially observed that people with methamphetamine dependence who started using the drug in adolescence have higher antisocial beliefs and poorer emotion recognition than those who started use during adulthood. Since we could not draw causality from these findings (i.e., whether adolescent methamphetamine use caused greater social cognition deficits, or social cognition deficits led to early onset of methamphetamine use), we subsequently conducted the mice studies to ascertain the effects of methamphetamine exposure on social cognition and aggression, and dopaminergic function during adolescence relative to adulthood. Since, beyond adolescence, young adulthood is the period of highest onset of methamphetamine use (Australian Institute of Health and Welfare, 2020; European Monitoring Centre for Drugs and Drug Abuse, 2021), the rodent study administered methamphetamine at two different stages: adolescence and young adulthood.

2. Methods

2.1. Human study

2.1.1. Participants

We tested 95 participants with methamphetamine dependence recruited through treatment centres in metropolitan Melbourne (Australia) and 49 controls recruited via

community advertisement in the same area (details reported in Fitzpatrick et al., 2020). We used the World Health Organization's definition of adolescence (i.e., between 10 and 19 years of age) to classify participants with methamphetamine dependence as Adolescent Onset (n = 38 / 7 female) or Adult Onset (n = 57 / 13 female). Participants with no history of methamphetamine use (n = 49 / 12 female) formed the healthy comparison group (henceforth, Controls). Inclusion criteria for the methamphetamine group included: aged 18 to 55 and having a primary diagnosis of methamphetamine dependence, measured with the Structured Clinical Interview for the DSM-IV (SCID-IV; First, M. B., Spitzer, R.L, Gibbon M., and Williams, 2002). The methamphetamine dependent group were also required to be abstinent from all drugs for a minimum of 2 days and a maximum of 21 days to minimise the impact of residual drug effects (lower bound) and inter-individual variability in the duration of methamphetamine abstinence (higher bound) on the assessments. Duration of abstinence was assessed using the Timeline Followback interview (Sobell et al., 2006), which is a well-validated and reliable measure of recent drug use (Donovan et al., 2012). Methamphetamine dependent participants were excluded if they met the DSM-IV criteria for dependence on any other substances other than methamphetamine, excluding tobacco, alcohol or cannabis. All participants were excluded during the initial semi-structured interview if they reported a loss of consciousness >30 minutes, a history of bipolar, schizophrenia or other psychotic disorders, an intellectual disability, or a neurological condition. Substance-induced psychotic symptoms were not exclusionary, as they are common and inherent manifestations of methamphetamine dependence (Arunogiri et al., 2020). Controls were also excluded if they had ever used methamphetamine or met the diagnostic criteria for any substance-related disorder. Participants in the control group were allowed to have regular tobacco smoking (we did not formally assess nicotine dependence), but could not meet criteria for alcohol or cannabis dependence.

2.1.2. Procedures

Recruitment took place between April 2015 and February 2017. Eligible individuals who were entering treatment for methamphetamine use were first identified by their clinicians and referred to the study. Potential participants were screened by the research team for eligibility prior to consent. All participants were assessed in a single session that lasted approximately 180 min (methamphetamine dependent group) and 90 min (controls); time difference due to extra time needed to complete clinical measures (e.g., SCID) in participants with methamphetamine dependence. Participants received $20 (AUD) as compensation for participating. The Eastern Health Human Research Ethics Committee approved the study (E52/1213).

2.1.3. Measures

2.1.3.1. Drug use and other clinical measures

Clinical interview. Two researchers with postgraduate training in clinical psychology conducted the SCID-IV to ascertain diagnosis of methamphetamine dependence / other substance-related disorders and exclusionary comorbidities. In addition, they conducted a semi-structured interview (Interview for Research on Addictive Behaviors; Verdejo-Garcia et al., 2005) to determine the age of onset of methamphetamine use, as well as lifetime amount / frequency and duration of use. To ensure adequate recollection of retrospective information, interviewers adopted an open non-judgmental approach, reemphasised confidentiality, and used well-validated memory prompts (Donovan et al., 2012).

Severity of Dependence Scales for methamphetamine, Alcohol and Cannabis (SDS; Gossop et al., 1995). A five question self-report measure that assesses the degree of an individual's level of dependence on a substance. We used it for methamphetamine (primary drug), alcohol and cannabis (other drugs used). Participants rated their answers on a four-

point scale (from ‘Never/almost never’ to ‘Always/nearly always’); higher scores represent a higher degree of dependence.

Timeline Followback (TLFB; Sobell et al., 1996). A well-validated interview to estimate amount/frequency of substance/s use in the last month before treatment using a calendar and other memory aids (e.g. birthdays, holidays).

Brief Psychiatric Rating Scale (BPRS; Lukoff et al., 1986). A dimensional measure of psychosis, based on a structured interview conducted by a trained interviewer. Each item incorporates a judgement of symptom frequency, severity and level of impact on function. Interviewers rate each item between 1-7 on the basis of severity, with 1 being “not present”, to 7 being “extremely severe”. The dependent variables were the total scores of the positive symptom items of suspiciousness, hallucinations and unusual thought content (ranging from 3-21).

2.1.3.2. Personality and social cognition measures

Personality Beliefs Questionnaire – Short Form (PBQ-SF; Fournier et al., 2012). A 65-item self-report measure of personality beliefs underlying social interactions associated with personality disorders. It comprises 10 subscales that assess: paranoid, schizoid, antisocial, borderline, histrionic, narcissistic, avoidant, dependent, obsessive-compulsive, and passive-aggressive beliefs. We were specifically interested in beliefs that have been previously associated with social cognition measures, i.e. Antisocial, Paranoid and Passive-aggressive (Albein-Urios et al., 2019; Hanegraaf et al., 2020). Example items include “Force or cunning is the best way to get things done” (Antisocial); “If people act friendly, they may be trying to use or exploit me” (Paranoid); “Authority figures tend to be intrusive, demanding, interfering, and controlling” (Passive-aggressive). Items are scored on a 4-point Likert-type scale ranging from 0 (“I don’t believe it at all”) to 4 (“I believe it totally”). Z-

scores for each subscale are computed according to the formula provided in the PBQ-SF scoring key (Butler et al., 2007). All items are scored in the same direction: higher scores indicate increasing levels of personality dysfunction.

Ekman Faces Test (EFT) (Young et al., 2002). A test of participants' ability to decode others' emotions based on their facial expressions (i.e., emotion recognition) and is a well-validated measure of social cognition. Sixty photographs expressing one of six basic emotions at 100% intensity (anger, fear, happiness, surprise, sadness and disgust) were presented individually on a black background for 5s followed by a blank screen where participants were instructed to choose the emotion that best described the picture using a forced-choice paradigm. The task used stimuli from the Facial Expressions of Emotion: Stimuli and Tests (FEEST, Young et al., 2002), and included ten presentations for each emotion displayed by 10 people (6 female, 4 male). Total scores range from 0 – 60, where higher scores represented better facial emotion recognition.

2.2. Rodent study

2.2.1. Animals, drugs and reagents

From a total of 137 male mice of the OF1 strain acquired from Charles River (Barcelona, Spain): 58 (30 adolescent and 28 young adult) were used for behavioural studies, and 79 (39 adolescent and 40 young adult) for biochemical studies. Adolescent animals were 21 days old and young adult animals 42 days old on arrival at the laboratory, and were housed in groups of five, under standard conditions (cage size 28x28x14.5cm), for eight days prior to initiating the experimental feeding schedule, at a constant temperature (21±2°C), with a reverse light cycle (white lights on 19:30 -7:30h). Food (standard diet) and water were available ad libitum in all the experiments (except during behavioural tests). Mice were

manipulated at the same time on each test day to minimize inter-day variability. All procedures involving mice and their care complied with relevant regulations: Directive 2010/63/EU of the European Parliament and the council of September 22, 2010. The Animal Use and Care Committees of the University of Valencia and the University of Barcelona approved the study. Methamphetamine hydrochloride was synthesized in racemic form by EE's group in the University of Barcelona (Spain). Primary mouse monoclonal antibodies against tyrosine hydroxylase (TH) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were purchased from BD Biosciences and Merck Millipore, respectively. Peroxidase-conjugated anti-mouse secondary antibody was from GE Healthcare.

2.2.2. Design and procedures

Supplementary Figure S1 displays the full design including all manipulation checks (e.g. pre-pulse inhibition, motor activity and anxiety) – see description in *Supplementary Information* and results in *Figures S2-S3* and *Table S1*. Methamphetamine (4 mg/kg) or saline (5 ml/kg) were injected intraperitoneally to mice once a day for 10 consecutive days. This dose was selected based on previous studies in order to avoid the well-known methamphetamine-induced neurotoxicity (Cadet et al., 2003; Isao and Akiyama, 2004; Miczek and O'Donnell, 1978; Sokolov and Cadet, 2006). The selected treatment schedule models a period of continuous drug use, which mimics the pattern of regular methamphetamine use typically observed during adolescence in epidemiological studies (Dhein et al., 2018), although it is not supposed to produce a marked neurotoxicity (Isao & Akiyama, 2004). Next, they were housed in their home cages until commencement of behavioural testing of social interaction (between 11 and 17 days after last methamphetamine or saline administration). Mice were sacrificed by cervical dislocation after 10 or 21 days of last methamphetamine or saline administration to obtain the corpus striatum for biochemical analyses.

2.2.3. Behaviour and social cognition measures

Social interaction. The social interaction test involves an interaction between an experimental animal and a standard opponent in a neutral cage (61 × 30.5 × 36 cm) for 10 min following 1 min of adaptation prior to the encounter. Standard opponents were rendered temporarily anosmic by intranasal lavage with a 4% zinc sulfate solution 1 day before testing (Smoothy et al., 1986). This kind of mouse does not outwardly provoke the experimental subject or defend itself, as it cannot perceive the pheromone that cues aggressive behaviour in mice with a normal sense of smell (Brain, 1981; Mugford and Nowell, 1970). Behaviour was videotaped under white illumination. The videotapes were analysed using a custom-developed program (Brain et al., 1989) that facilitates estimation of times allocated to different broad functional categories of behaviour each of which is characterized by a series of different postures and elements. The following behaviours were analysed in the study: time in social investigation and aggressive behaviours (threat and attack). We have extensively validated this paradigm in our laboratory; a more detailed description can be found in (Rodríguez-Arias et al., 1998).

Aggression. The resident-intruder test comprises an episode of social defeat lasting 25 min. Includes three phases that began by placing the experimental animal (intruder) in the home cage of the aggressive opponent (resident) for 10 min. During this initial phase a wire mesh wall protected the intruder, although this wall permitted social interaction and species-typical threats from the resident (Covington and Miczek, 2001). In the second phase, the wire mesh was removed, and a 5-min period of confrontation began. In the third phase, the wire mesh was restored for a further 10 minutes to allow social threats from the resident. We video-recorded and ethologically analysed the second phase to assess aggressive behaviours (threat and attack) and avoidance/defensive behaviours in the intruder.

2.2.4. Striatum biochemical measures

Striatal synaptosomes and [3H]DA uptake. The striatums were dissected out on ice, weighed and homogenized in 60 volumes (W/V) of ice-cold homogenization buffer (5 mM Tris-HCl and 320 mM sucrose) using a Teflon/glass homogenizer. After a first centrifugation at 1000 x g for 10 min., the supernatant was subjected to 13000 x g for 30 min. to obtain the P2 synaptosome fraction. The P2 pellet was resuspended in 0.5 ml of Hank's Balanced Salt Solution (HBSS, Biological industries, Inc.) supplemented with 5.5 mM glucose, 20 mM HEPES sodium, 10 µM pargyline and 1 mM ascorbic acid (pH 7.4). This buffer was also used as the uptake reaction buffer. The protein concentration was determined using the Bio-Rad Protein Reagent (Bio-Rad Labs., Inc., Hercules, CA, USA).

For [^{3}H]DA uptake, 0.1 ml of synaptosome suspension was added to 0.125 ml of uptake buffer and warmed at 37 °C. Then, 0,025 ml of [^{3}H]DA (final concentration 2 nM) was added to start the incubation for a further 5 min. at 37 °C. The uptake reaction was stopped by rapid filtration of the content of the tubes through Whatman GF/B glass microfiber filters pre-soaked in 0.5% polyethyleneimine solution followed by three washes of the tubes and filters with ice-cold buffer. Filters were placed in vials containing scintillation cocktail (Ultima Gold MV, Perkin Elmer, MA, USA) and the trapped radioactivity was measured by liquid scintillation spectrometry. Non-specific uptake was determined at 4 °C in parallel samples containing 100 µM cocaine, and specific [^{3}H]DA uptake was calculated subtracting non-specific uptake values from those of total uptake. Specific uptake values were corrected by dividing by the protein concentration and expressed as percentage of uptake with respect to controls (saline-treated). Each experiment was run in duplicate tubes.

[^{3}H]Spiperone binding. [^{3}H]Spiperone binding was used to quantify D2-like receptors (Camarasa et al., 2006). Homogenized striatal samples were centrifuged three times at 15000 x g in order to remove endogenous ligands and obtain the crude membrane fraction. This

final pellet was resuspended in a binding buffer consisting in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 0.01% ascorbic acid, 0.007% bacitracin, 0.05 mM PMSF, 0.1 mM EDTA, 10 µM pargiline and 0.1 µM, ketanserine (pH=7.5). Protein concentrations were assessed as cited above. Binding was carried out in glass tubes (final volume, 3 ml) containing 200 µg of protein and [^{3}H]spiperone (final concentration 200 pM) which was added at the start of an incubation of 3 h at 25 °C. The binding reaction was stopped by rapid filtration as described previously for uptake experiments, and the radioactivity trapped in the filters was also measured by liquid scintillation. Non-specific binding was assessed in parallel tubes containing 2 µM butaclamol and was subtracted from total binding values. The resulting specific binding values were expressed as a percentage of binding with respect to control (saline-treated) values.

Monoamine oxidase (MAO) activity. MAO activity was assessed on the cytoplasmic fraction obtained as described for Western blot experiments, using the MAO activity assay kit (Merck, Darmstadt, Germany). In this assay, MAO reacts with p-tyramine, a substrate for both MAO-A and MAO-B, resulting in the formation of H_2O_2, which is detected by a fluorometric method. The remaining MAO-B activity was determined in control wells containing 5 µM clorgyline to inhibit MAO-A activity and was subtracted from the total MAO activity in the sample well to obtain neat MAO-A activities. Fluorescence was determined in a fluorescence multi-well plate reader using excitation/emission wavelengths of 530/590 nm. MAO-A activity was expressed as percentage with respect to control (saline-treated) values.

Tyrosine hydroxylase levels. Striatal samples were homogenized with a sonicator in cold homogenization buffer (5 mM Tris-HCl and 320 mM sucrose) containing protease inhibitors and centrifuged (1000 x g, 15 min., 4 °C) to obtain the cytoplasmic fraction (supernatant) from which an aliquot was removed for Western blotting. The remaining of the

samples was mixed and subjected to further centrifugations to obtain the crude membranes for binding experiments (see *[^{3}H]Spiperone binding* section in *Supplementary Information*). The protein content was determined as cited above and then adjusted to 0.5 mg/ml in sample buffer (62.5 mM Tris–HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 5% (v/v) 2-β-mercaptoethanol, 0.05% bromophenol blue, final concentrations), boiled for 5 min and loaded onto a 10% polyacrylamide gel. After electrophoresis at 120 V during 1.5 h, proteins were transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-P; Millipore, Billerica, USA). The membranes were washed in TBS-T (0,05% Tween-20 in Tris-Buffered solution (TBS)) and then blocked for 1 h in 5% defatted milk in TBS-T. Membranes were incubated overnight at 4ºC with a primary mouse monoclonal antibody against TH (BD Biosciences, Franklin Lakes, NJ, USA) diluted 1:10000. After washing 3 times for 5 minutes in TBS-T, membranes were incubated with a peroxidase-conjugated anti-mouse antibody (GE Healthcare, Buckinghamshire, UK) diluted 1:2500 in TBS-T. Immunoreactive protein was visualized using a chemiluminescence-based detection kit (Immobilon Western, Millipore, USA) and a Bio-Rad ChemiDoc XRS imaging system (Bio-Rad Labs., Inc., Hercules, CA, USA). Immunodetection of GAPDH (mouse monoclonal antibody, 1:5000) served as a loading control. Quantification of TH was corrected by dividing each value by that of its matching loading control (GAPDH) and expressed as percentage with respect to the control group (saline-treated).

2.3. Statistical Analyses

Power analyses: The human study was part of a larger project, which was powered for case-control comparisons between people with methamphetamine dependence and controls to detect medium effect size differences with 80% power and .05 alpha (Fitzpatrick et al., 2020). As in rodent studies we performed several ANOVAs for the statistical analysis, we chose to calculate partial eta-squared (η_p^2) for each comparison.

Human study: Data for at least one dependent measure was present for 36 / 55 / 43 participants with Adolescent Onset / Adult Onset / Controls respectively, and used for analyses. We used one-way analyses of variance (ANOVAs) to test between-group differences (participants with Adolescent Onset versus Adult Onset versus Controls) in background characteristics and the dependent measures of interest (i.e. Antisocial, Paranoid and Passive-aggressive beliefs and EFT scores). ANOVAs that yielded significant results ($p < 0.05$) were followed up with Least Significant Differences (LSD) tests, which are optimal for paired comparisons involving three groups (Cardinal and Aitken, 2013). We were specifically interested in differences between participants with Adolescent Onset and Adult Onset of methamphetamine use. In addition to the main analyses, we conducted a series of sensitivity analyses to rule out a significant influence of a number of potential confounders. To ensure that the effects of age of onset on social cognition were not confounded by amount / frequency or duration of methamphetamine use, we conducted Spearman correlation analyses between severity (product of: typical amount x frequency) and duration scores derived from the Interview for Research on Addictive Behaviors and social cognition measures. Furthermore, since some participants with methamphetamine dependence (unlike controls) had history of alcohol and cannabis use, we also included alcohol and cannabis comorbidities in sensitivity analyses of covariance. Likewise, since sex can potentially influence methamphetamine effects on neurocognition as a function of age / developmental stage, we conducted additional univariate ANOVAs to examine sex and sex by methamphetamine age of onset interaction effects on social cognition measures.

Rodent study: We used two-way ANOVAs including Age (Adolescent, Adult) and Treatment (Vehicle, methamphetamine) on behavioural and biochemical dependent measures using SPSS v26. We were specifically interested in Age x Treatment interaction effects, to assist with the interpretation of the effects of age of onset in humans (i.e. if they could be

attributed to direct neuroadaptive effects of methamphetamine use during adolescence). When the interaction effect was significant ($p<0.05$), we conducted multiple comparison corrected post hoc Bonferroni tests to disambiguate effects. Every set of results was tested in the calculator QuickCalcs of GraphPad software, which performs Grubbs' test (extreme studentized deviate method) to control for possible outliers. Data from biochemical experiments analysis were expressed as mean ± SEM and were normalized with 100% defined as the mean of the replicates in the control group.

3. Results

3.1. Human study

3.1.1. Background and clinical characteristics

Table 1 displays the results. The three groups had similar socio-demographic characteristics (i.e., sex, verbal IQ and socioeconomic status) although they differed on age (participants with Adolescent Onset were on average 4 to 6 years younger than Controls and participants with Adult Onset respectively), but note that age was not correlated with any of the dependent measures (all *p*s>0.09). Participants with Adolescent Onset and Adult Onset had similar severity of methamphetamine dependence (SDS-M), as well as alcohol and cannabis dependence (SDS-A and SDS-C), and BPRS measures of psychotic symptoms. They also showed very similar patterns of methamphetamine use in the last month before starting treatment (TLFB).

(TABLE 1)

3.1.2. Social cognition measures

Dysfunctional beliefs. *Table 2* displays the results. We found significant between-group differences in the three beliefs of interest: Antisocial, Paranoid and Passive-aggressive ($p<0.001$). However, differences between participants with Adolescent Onset and Adult Onset of methamphetamine use were specific to the Antisocial domain ($p<0.046$, Cohen's $d=0.42$); participants with Adolescent Onset had higher antisocial beliefs than those with Adult Onset. For Paranoid and Passive-aggressive beliefs, there were differences between Controls and both participants with Adolescent Onset and Adult Onset of methamphetamine use (*ps* <0.001), but no differences between the methamphetamine groups (*p*s >0.40).

Emotion recognition. Table 2 displays the results. We found significant between-group differences in Total emotion recognition ($p=0.027$), and in the critical comparison between participants with Adolescent Onset and Adult Onset ($p=0.031$, Cohen's $d=0.44$). There were no significant differences between participants with Adult Onset and Controls ($p=0.54$). We also explored discrete emotion recognition scores, and we found between-group differences in the recognition of Anger ($p=0.015$); participants with Adolescent (but not Adult) onset had lower recognition of expressions of Anger ($p=0.004$, Cohen's $d=0.61$). Interestingly, this pattern was driven by more incorrect identifications of expressions of Anger as Sadness (Cohen's $d=0.53$ for the comparison between participants with adolescent onset and controls).

(TABLE 2)

Sensitivity analyses. The results from the correlations between duration and severity of methamphetamine use and social cognition measures were not statistically significant (*Rho* range -.009, -.111), suggesting that the contribution of age of onset to social cognition deficits is independent of the effect of length of use or cumulative use of methamphetamine over the years. The inclusion of alcohol and cannabis comorbidities in analyses of covariance for

social cognition measures did not either change results; the effects of methamphetamine age of onset remained significant, and alcohol and cannabis covariates did not significantly contribute to any of the dependent variables (p-values >0.08 <0.99). We also conducted additional analyses of variance to examine the effect of sex. We did not find significant effects of sex, or the sex by age of onset interaction in any of the dependent measures (p-values >0.09 <0.85).

3.2. Rodent study

3.2.1. Behavioural measures

Social Interaction. Results are displayed in *Figure 1.* For time spent in Social Investigation, we found a significant Age x Treatment interaction [$F(1,54)=5.083$; $p=0.028$; $\eta_p^2=0.08$]. This interaction indicated that methamphetamine disrupts the adolescent-specific behaviour of spending more time in social investigation: saline-treated adolescent mice spent more time in social contacts than young adults ($p<0.001$), and methamphetamine specifically decreased this behaviour in adolescent animals ($p<0.001$). For the remaining dependent measures, only a general effect of treatment was found, inducing methamphetamine the same effects in young adults and adolescent mice (*Table S2*).

(FIGURE 1)

Aggression. Results for intruder mice in the Resident-Intruder Test are displayed in *Figure 2.* For time spent in Threat and Attack, we found a significant Age x Treatment interaction [$F(1,53)=4.088$; $p=0.048$; $\eta_p^2=0.072$]. This interaction indicated that methamphetamine induced a greater increase in defensive aggression in young adults relative to adolescents: methamphetamine-treated young adults had greater aggression than both saline-treated ($p<0.001$) and methamphetamine-treated adolescents ($p<0.01$). Although

methamphetamine also increased this type of aggression in adolescent mice, it did not reach statistical significance (*Table S3* and *Figure S4*).

(FIGURE 2)

3.2.2. Striatum biochemical measures

Although we did not find significant Age x Treatment interaction effects on DA uptake, D_2 levels, or MAO activity (details in Supplementary Materials: *Figures S5-S7*), there was a significant interaction on TH levels 21 days after last methamphetamine exposure [$F(1,16)= 4.936$; $p=0.05$]. Mice treated with methamphetamine during adolescence showed a significant 40% increase in TH with respect to those treated during young adulthood ($p<0.01$ for the Bonferroni test; *Figure 3A*). Interestingly, we observed that TH levels were significantly *decreased* in individuals exposed to methamphetamine during adolescence when measured 10 days after treatment, although the age x treatment interaction effect did not reach statistical significance (*Figure 3B*). In other words, just at the end of treatment, adolescent mice showed a deficit in the expression of TH induced by methamphetamine, which reversed after 21 days of withdrawal. That is, following this treatment schedule, the expression of TH in adolescents is dependent on methamphetamine intake, which is not seen in young adults. Regardless the lack of significant interaction between factors, both saline- and methamphetamine-treated adolescent mice showed lower densities of dopamine D2 receptors than young adults after 10 days of withdrawal (Supplementary Fig. S6). Such densities restored after 21 days of withdrawal but, at that time point, the adolescent mice had significantly lower MAO-A activity than their respective young adult groups.

(FIGURE 3)

4. Discussion

We found social cognition deficits associated with methamphetamine use during adolescence in humans and mice. Specifically, people with methamphetamine dependence who started use in adolescence had more antisocial beliefs concerning social interactions and worse emotion recognition, particularly poorer anger recognition, than those who started use in adulthood. The complementary rodent findings showed that methamphetamine exposure during adolescence reduced social investigation during social interaction. Therefore, methamphetamine administered during adolescence affected only spontaneous social interaction in a neutral environment, but did not change reactive behavior when the experimental mouse was exposed to an aggressive opponent. Both human and mice measurements are well-validated indicators of social cognition deficits, but no studies had previously interrogated them in an integrated translational study using similar drug exposures. Altogether, our findings suggest that methamphetamine use during adolescence may directly deteriorate specific aspects of social cognition; that is, adolescence constitutes a stage of increased sensitivity to the deleterious effects of methamphetamine use on social cognition. We should nonetheless acknowledge the inherent limitations of rodent models of social cognition, which cannot speak to the thinking processes underlying antisocial beliefs or the nuances of human facial emotional decoding. With regard to the neurochemical underpinnings of social cognition deficits, we found that methamphetamine specifically impacted striatal tyrosine hydroxylase (TH) during adolescence. Methamphetamine-induced dysregulation of TH has been previously reported using more neurotoxicity-sensitive strains and only slightly higher doses of the drug (Biagioni et al., 2019). Although we showed that TH dyregulation effects recovered after withdrawal, they are still potentially clinically meaningful, as TH alterations may disproportionately rebound after subsequent drug use and

since withdrawal episodes are a known contributor to cognitive impairment in people with substance use disorders (Loeber et al., 2009, 2010).

Our reverse translational approach suggested that the social cognition deficits of people with adolescent onset of methamphetamine use (i.e., antisocial beliefs and emotion recognition) could be at least partly explained by methamphetamine-related disruption of adaptive social investigation as observed in the rodent model. Previous studies have linked antisocial personality traits with cognitive biases reflecting decreased social investigation in humans. Specifically, antisocial traits were associated with reduced exploration of social communication cues from others' faces (Boll and Gamer, 2016). We also showed that adolescent onset of use specifically affects the recognition of anger, but not in the way that is traditionally interpreted as a hostility bias (i.e. more misclassification of emotions as anger (Hanegraaf et al., 2020; Martin et al., 2006)), but rather as a "freeze" bias (i.e. misclassification of anger as sadness). In addition to aligning with our reduced social investigation interpretation, this finding may also partially explain heterogeneous findings previously reported in regards to methamphetamine use and anger recognition (Hanegraaf et al., 2020), by demonstrating the impact of age of onset in inter-individual differences. Previous studies have similarly found that earlier age of onset is associated with deficits in emotion recognition and empathy among users of other stimulants such as cocaine, and these effects seem to be underpinned by functional alterations in amygdala function (Crunelle et al., 2015; Preller et al., 2014), which we did not measure here. Reduced social investigation in our rodent model was underpinned by overexpression of TH in the striatum, which in previous studies has been associated with decreased social interaction (Baek et al., 2014; Lampert et al., 2017).

In fitting with our approach, whereby we used the rodent model to examine drug-induced mechanisms underpinning social cognition deficits in humans, we interpreted our

overall findings in the context of drug-related effects. However, it is also possible that predispositions associated with reduced social engagement, such as interpersonal avoidance or low social motivation traits predispose adolescents to stimulant use (Belcher et al., 2014), or that both paths interact synergistically. Preclinical research has consistently shown that volitional social interaction during adolescence prevents development and escalation of substance use (Venniro et al., 2018). A similar path has been described in humans, in which adolescent social withdrawal is a risk factor for development of substance-related problems (Polek et al., 2018). Our results demonstrating disruption of personality features that emerge during adolescence in humans (i.e. antisocial beliefs), and social investigation behaviours relevant to gain "adult" social dominance in rodents, may suggest that methamphetamine use may disrupt adaptive development of social cognition skills.

Further, our rodent findings suggest that young adult age of onset of methamphetamine use is associated with increased aggression in comparison to adolescent age of onset. Indeed, rodents receiving methamphetamine during young adulthood displayed increased aggressive behaviour in comparison to adolescent rodents. This was seemingly consistent with the results from our human study, which showed that people with methamphetamine dependence and adolescent age of onset perceived angry faces as less hostile than those with adult age of onset. These results are interesting as they show that one of the most concerning aspects of methamphetamine-related clinical presentations (i.e. aggression, violence) is not specifically associated with early use. Rodent findings are likely due to developmental differences in testosterone levels (Bell, 2018). Thus, in future studies, it would be interesting to investigate the influence of baseline testosterone levels, and DA x testosterone interactions in determining methamphetamine-related aggression in humans (Rosell and Siever, 2015). However, another feasible explanation for such differences in aggression depending on the age of methamphetamine consumption onset might be given by

the significant differences in striatal D2 receptors that we found in mice. Adolescent mice showed significantly lower levels of D2 receptors than young adults (Fig. S 6), and previous reports have related lower D2 receptor population with reduced aggression in a non-aggressive mouse strain (Couppis et al., 2008).

This study has important strengths including novel investigation of social cognition using a reverse-translational design. There are also important limitations inherent to human-mice translation and related interpretation. Specifically, human findings are based on retrospective assessment of age of onset of methamphetamine use in a single sample, whereas the rodents were administered methamphetamine during adolescence and young adulthood in independent groups. A critical limitation in the rodent study is the age of onset, as the young adult group received the methamphetamine doses on PND50, which is considered as late adolescence – early adulthood (Madsen and Kim, 2016). Future studies should consider administering methamphetamine doses at older ages and in different patterns. The rodent study does not allow an analysis of the pattern of drug intake that reflects the human study, as all mice were given the same number of injections. However, it shows that with the same exposure to methamphetamine, adolescent animals showed more behavioral changes than those exposed during adulthood. In addition, although the measures used in humans and animals tap into similar constructs, they have very different features and levels of analysis; for instance, it is not possible to assess personality biases in animals. Furthermore, there are factors, other than adolescent-specific neuroadaptations, which may have influenced the differential effects of methamphetamine on adolescent social cognition in mice. One such factor is the potential differences in methamphetamine metabolism across age, as evidenced by previous studies (Kokoshka et al., 2000; Miller et al., 2000). Moreover, hormonal development likely played a key role in animal models, and although we ascribe some of the

methamphetamine-specific effects in young adult mice to testosterone levels we did not directly measure them.

Notwithstanding limitations, this cross-species study offers novel and potentially clinically relevant findings on the impact of adolescent methamphetamine use on social cognition – a key set of processes to ensure adaptive social and emotional functioning.

5. Conclusions

In humans, adolescent onset of methamphetamine use is associated with elevated antisocial beliefs and worse emotion recognition. In mice, methamphetamine administration during adolescence generated specific detrimental effects on social investigation, as well as abnormalities in striatal tyrosine hydroxylase levels. Altogether, our reverse translational findings suggest that adolescent methamphetamine use deteriorates social cognition skills, potentially contributing to social disadvantage among methamphetamine-dependent users.

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Table 1. Participant Demographics and Comparisons Between Groups

	METH Adolescent Onset (*n*=38)		METH Adult Onset (*n*=57)		Controls (*n*= 49)			
	M	*SD*	*M*	*SD*	*M*	*SD*	Statistics	*p*
Age (years)	27.14	5.48	33.97	7.39	31.18	8.59	*F* (2,141) = 9.72	<.001
Sex (% male)	82		77		76		Chi = 0.48	.79
Verbal IQ*	46.76	7.44	47.89	8.01	48.75	8.32	*F* (2,140) = .66	.52
SES	7.05	2.31	7.11	2.45	7.94	1.59	*F* (2,139) = 2.48	.09
Age of onset METH use^	17		26					
Daily METH use (grams)	.85	.72	.68	.61			*t* (93) = 1.24	.22
METH use in last month (days)	23.54	9.68	22.96	9.26			*t* (88) = .29	.78
SDS METH	10.39	3.53	11.38	3.01			*t* (93) = -1.46	.15
SDS Marijuana	2.92	4.37	2.27	4.19			*t* (92) = .73	.47
SDS Alcohol	2.11	3.95	1.79	3.07			*t* (92) = .44	.66
BPRS suspicion	2.34	1.57	2.11	1.06			*t* (53.95) = .77	.45
BPRS hallucinations	2.17	1.54	2.26	1.23			*t* (87) = -.30	.77
BPRS unusual thought content	1.80	1.28	1.69	1.20			*t* (87) = .43	.67

Note. *T-scores from the Wechsler Abbreviated Scale of Intelligence. ^Median. METH = Methamphetamine; SES = Socioeconomic Status; Adol = adolescent age of onset; Con = controls; SDS = Severity of Dependence Scale; BPRS = Brief Psychiatric Rating Scale

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Table 2. Group Differences in Dysfunctional Personality Beliefs and Emotion Recognition

	METH Adolescent Onset (*n*=38)		METH Adult Onset (*n*=57)		Controls (*n*= 49)				
	M	*SD*	*M*	*SD*	*M*	*SD*	Group ANOVA	*p*	Comparison
PBQ Antisocial	3.39	1.44	2.84	1.23	2.19	1.14	*F* (2,130) = 8.91	<.001	Adol > Adults, Con
PBQ Paranoid	2.33	1.14	2.33	0.94	1.32	0.66	*F* (2,128) = 17.26	<.001	Adol, Adults > Con
PBQ Passive-Aggressive	2.18	0.83	2.04	0.72	1.36	0.76	*F* (2,130) = 13.55	<.001	Adol, Adults > Con
EFT Total	42.74	9.28	46.04	6.05	46.90	5.71	*F* (2,128) = 3.73	.03	Adol < Adults, Con
EFT Anger	6.38	2.40	7.16	1.61	7.69	1.87	*F* (2,128) = 4.37	.02	Adol < Adults, Con
EFT Disgust	6.56	2.49	6.80	2.13	7.21	2.11	*F* (2,128) = .86	.42	
EFT Fear	5.97	2.22	6.64	2.16	6.52	2.18	*F* (2,128) = 1.04	.36	
EFT Sadness	6.44	2.33	7.18	2.15	7.19	1.99	*F* (2,128) = 1.51	.23	
EFT Surprise	8.18	2.02	8.56	1.49	8.48	1.50	*F* (2,128) = .60	.55	

Note. METH = Methamphetamine; Adol = adolescent age of onset; Con = controls; PBQ = Personality Beliefs Questionnaire; EFT = Ekman Faces Task

Figure 1

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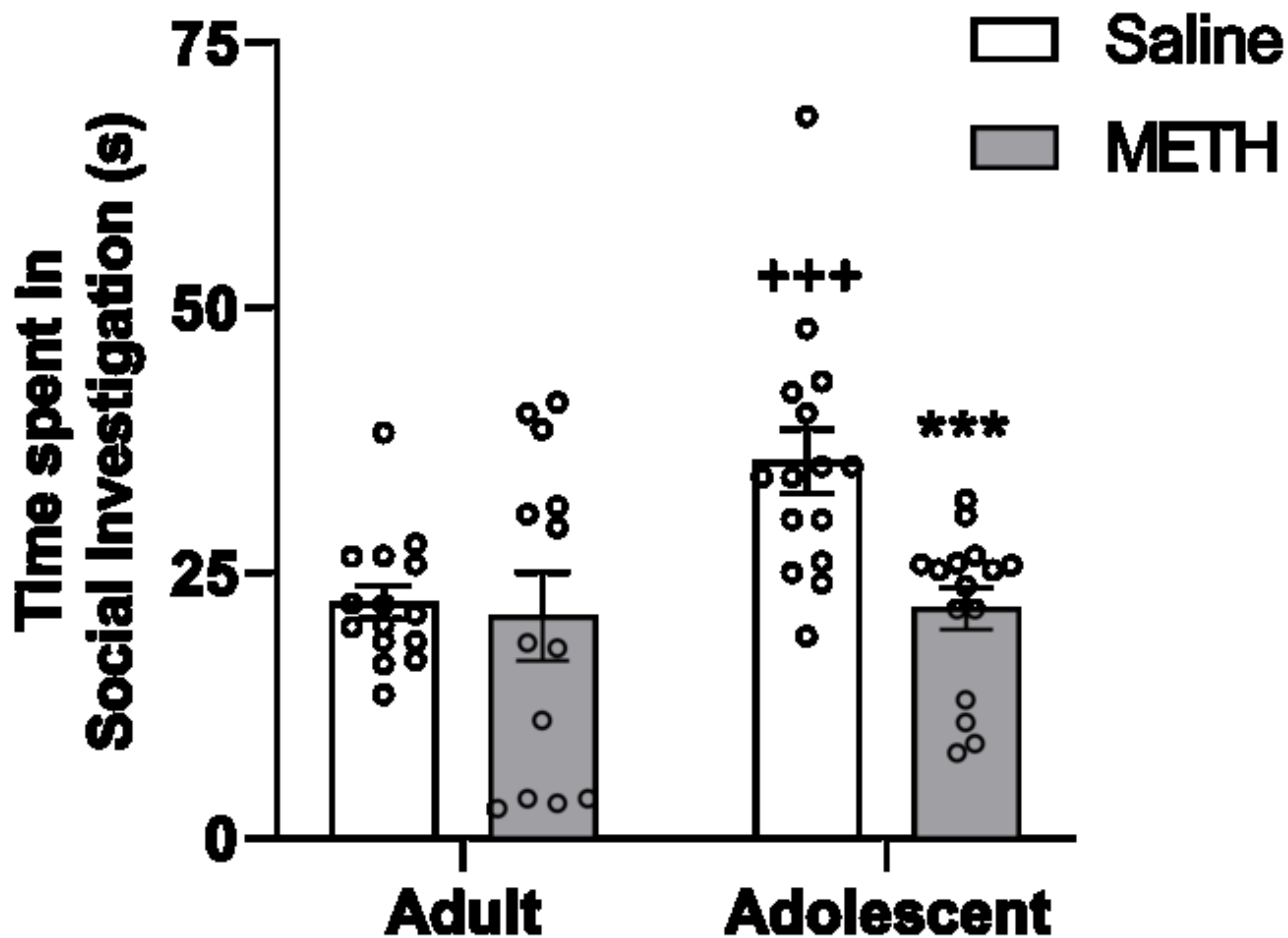

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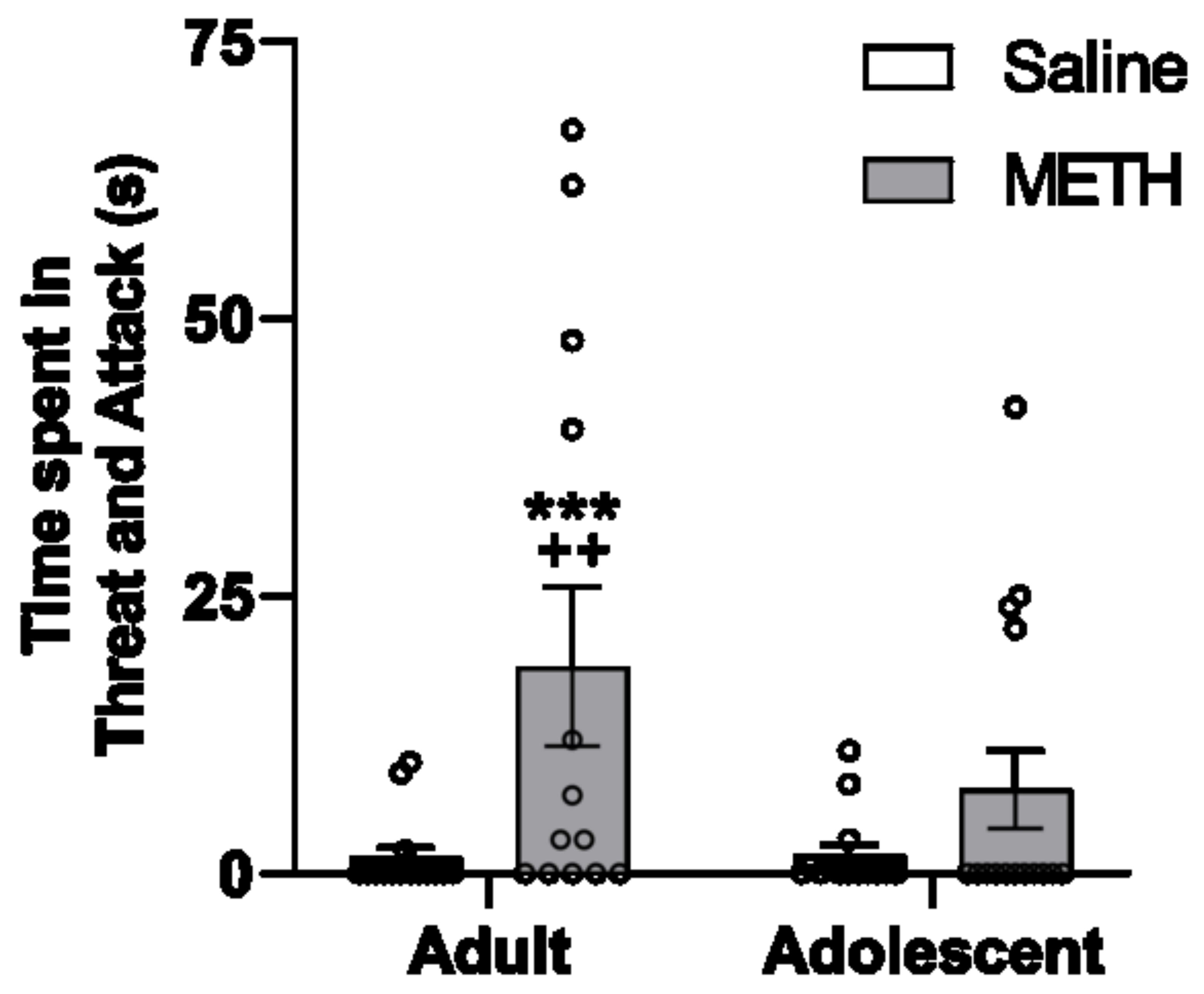

Figure 3

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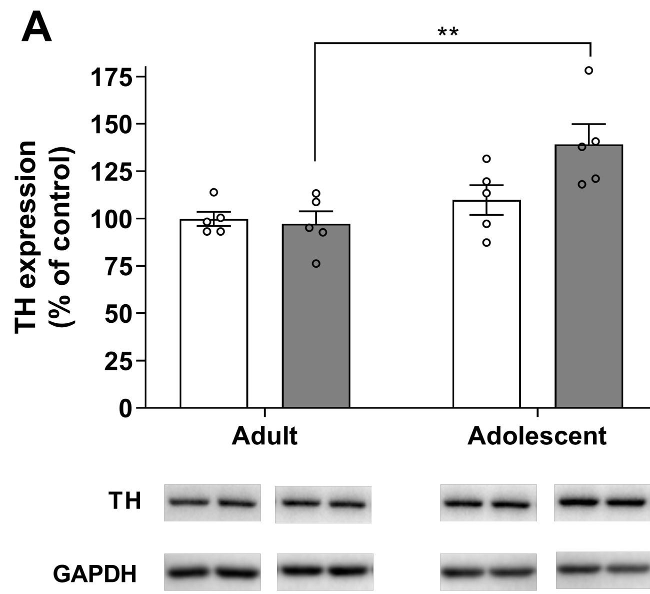
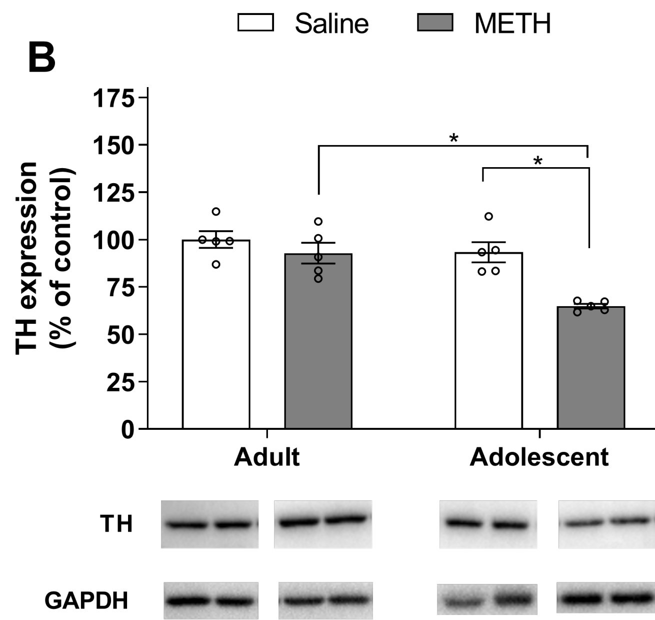
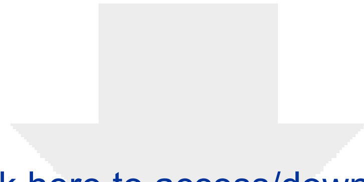


Saline METH



Author Contributions

AVG designed the study and wrote the first draft of the manuscript. LH conducted emotion recognition analyses. MCBG and MRA are responsible for the behavioural experiments in rodents. EE designed the biochemical study. RLA, DP and MG conducted biochemical assays. All authors contributed to the interpretation of the data and the final version of the paper. All authors approved the final manuscript.



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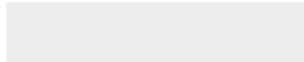

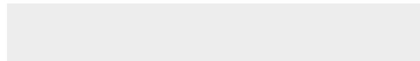

Funding and Disclosure

AVG is funded by an Australian Medical Research Future Fund, Next Generation of Clinical Researchers CDF2 Fellowship (MRF1141214). MRA is funded by Instituto de Salud Carlos III, Red de Trastornos Adictivos (RD16/0017/0007) and European Union, FEDER Funds “A way to build Europe”; EE is funded by Ministerio de Economia y Competitividad (grant number SAF2016-75347-R), Plan Nacional sobre Drogas (#2016I004). EE, RLA and DP belong to 2017SGR979 from Generalitat de Catalunya.

Conflict of interest

The authors declare no conflicts of interest.

Highlights

- Adolescent onset of meth use linked to poorer social cognition in humans
- Meth treatment during adolescent linked to poorer social interaction in mice
- Reverse translation suggest adolescent-specific meth effects on social cognition