Disruption of the ErbB signaling in adolescence increases striatal dopamine levels and affects learning and hedonic-like behavior in the adult mouse

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Received 21 February 2014; received in revised form 7 July 2014; accepted 24 September 2014

Abstract

The ErbB signaling pathway has been genetically and functionally implicated in schizophrenia. Numerous findings support the dysregulation of Neuregulin (NRG) and epidermal growth factor (EGF) signaling in schizophrenia. However, it is unclear whether alterations of these pathways in the adult brain or during development are involved in the pathophysiology of schizophrenia. Herein we characterized the behavioral profile and molecular changes resulting from pharmacologically blocking the ErbB signaling pathway during a critical period in the development of decision making, planning, judgments, emotions, social cognition and cognitive skills, namely adolescence. We demonstrate that chronic administration of the pan-ErbB kinase inhibitor JNJ-28871063 (JNJ) to adolescent mice elevated striatal dopamine levels and reduced preference for sucrose without affecting locomotor activity and exploratory behavior. In adulthood, adolescent JNJ-treated mice continue to consume less sucrose and needed significantly more correct-response trials to reach the learning criterion during the discrimination phase of the T-maze reversal learning task than their saline-injected controls. In addition, JNJ mice exhibited deficit in reference memory but not in working memory as measured in the radial arm maze. Inhibition of the pathway during adolescence did not affect exploratory behavior and locomotor activity in the open field, social interaction, social memory, and reversal learning in adult mice. Our data suggest that alteration of ErbB signaling during...
1. Introduction

Ligands of the ErbB receptors family are characterized by an epidermal growth factor (EGF) domain that is necessary to bind and activate the receptors (Falls, 2004). Numerous developmental processes in the central nervous system depend upon, or are regulated by, ErbB signaling, including myelination of peripheral nerves, interneuron migration, and glutamatergic synapse maturation (Iwakura and Nawa, 2013; Mei and Xiong, 2008). Among the ErbB receptors family, ErbB1, ErbB4 and their respective ligands EGF and NRG, have been identified as biological pathways potentially involved in the pathophysiology of schizophrenia because they modulate the dopaminergic, GABAergic, and glutamatergic systems. For example, in adulthood, infusion of NRG1 into the hippocampus increases levels of extracellular dopamine (DA) (Kwon et al., 2005), while perinatal exposure to EGF or NRG evokes overflow of extracellular striatal DA (Futamura et al., 2003; Kato et al., 2010a). Furthermore, both ErbB1 and ErbB4 are widely expressed in the mesolimbic area (Abe et al., 2009). Thus, manipulation of the pathway may trigger changes in the DA mesolimbic and mesocortical pathways, and provoke behavioral abnormalities relevant to cognition and social skills. In addition, recent rodent work from several laboratories suggests that NRG1 and ErbB4 may play important roles in modulating hippocampal and frontal cortical pyramidal neurons, and in local neuronal network activity in the form of gamma oscillation, possibly by signaling through GABAergic and dopaminergic neurotransmission (Andersson et al., 2012; Buonanno, 2010). Taken together, alteration in the ErbB signaling can affect a number of neural processes including the balance between excitatory and inhibitory transmissions, oscillatory activity, and synaptic plasticity, and therefore may influence the development of normal behavior. However, while the importance of ErbB signaling in neuronal activity is well established, little is known how these activities link to the development of normal adult behavior.

Perinatal administration of EGF or NRG resulted in abnormal social interaction and sensorimotor gating, while mice overexpressing the NRG1 type I isoform showed impaired spatial working memory, sensorimotor gating, and social behavior (Deakin et al., 2011; Yin et al., 2013). On the other hand, Shamir et al. (2012) recently reported that mice lacking the ErbB4 gene exhibited deficits in sensorimotor gating and impairment in acquisition of emotional memory. Deficits in sensorimotor gating, short-term memory, spatial memory, and social interaction were also reported in hypo morph NRG1 mice (Chen et al., 2008; O’Tuathaigh et al., 2007), and increases in NRG1 levels also lead to similar behavioral deficits (Yin et al., 2013; Luo et al., 2014). Together, these seemingly contradictory results suggest that unbalanced ErbB signaling is associated with the behavioral phenotype related to cognitive and social skills; however, the critical period (prenatal, neonatal or postnatal brain development) and the mode of alteration (gain or loss of function) are unclear and controversial.

Cognitive processing, including memory, flexibility, attention, social interaction and associative learning has been investigated in animal models with a high degree of validity (Papaleo et al., 2012; Kellendonk et al., 2009). To further investigate the role of ErbB signaling in the development of normal adult behavior in general and social and cognitive related behaviors, and to address these important issues, we blocked the pathway during a critical time in the development of social and cognitive skills—adolescence. During this period, in particular, frontal cortical brain regions and related neural circuitry such as oscillations are structurally remodeled and optimized (Steinberg, 2005; Yurgelun-Todd, 2007). A battery of behavioral tests related to learning, motivation, and social skills such as working memory, discrimination and reversal learning, social interaction, and hedonic behavior was performed to study the behavioral consequences of sub-chronic pharmacological blocking of ErbB signaling at adolescence on cognitive and social function in adulthood. This approach allows inhibition of the pathway for a short or long time, at a specific time frame, and at different stages of brain development (i.e postnatal). In addition and since we used the pan-ErbB inhibitor JNJ (see Section 2), pharmacological inhibition, unlike genetic manipulation, addresses how the entire ErbB pathway system, and not one of the family members, mediates normal adult behavior. We hypothesize that interfering with the ErbB signaling pathway during a critical period in cognitive and social skill development will alter cognitive processing and functioning in the adult mouse.

2. Experimental procedures

2.1. Animals and drug treatments

ICR (CD-1®) outbred male mice were purchased from Harlan Israel (Harlan, Rehovot, Israel). Animals were group-housed (unless it stated otherwise), and maintained on a 12 h/12 h light/dark cycle (lights on between 7:00 a.m. and 7:00 p.m.) with ad libitum access to food and water. Animals were treated in accordance with NIH Animal Welfare guidelines, and all procedures were approved by the Ort Braude College Animal Care and Use Committee. All the behavioral tests were performed during the light phase of the cycle between 7:00 a.m. and 7:00 p.m.

The ErbB signaling was blocked by JNU-28871063 (JNU; Tocris, Ellisville, MO), a potent and highly selective pan-ErbB kinase inhibitor that crosses the blood brain barrier (Emanuel et al., 2008), 5 mg/kg JNU-28871063 was dissolved in saline containing 5% (v/v) DMSO and administered intraperitoneally once daily for 14
days between postnatal day (PND) 24 and 48 (Spear, 2000). The JNJ dose was selected based on preliminary experiments analyzing dose-dependence (Supplementary Figure 1). Control group received injections of 5% DMSO dissolved in saline of the same volume. JNJ and saline were given in a volume of 0.1 ml per 10 g of mouse body weight.

2.2. Quantification of dopamine content

Total striatal DA content was determined 24 h after the last day of injections. Tissues were immediately frozen and stored at −80 °C until use. DA was measured by ELISA kit according to the manufacturer’s instructions (IBL-America, Minneapolis, MN). In brief, samples were homogenized in 0.01 N HCl, 1 mM EDTA, and 4 mM Sodium metabisulfite solution. Standards and controls were diluted up to 100 μl with distilled water; 100 μl of standards, controls, and samples were added to the extraction plate. After extraction and acylation, 90 μl of the calibrators, controls, and unknowns were added to the 2nd microtiter plate for an enzymatic conversion for 2 h at 37 °C. 100 μl of all supernatants were transferred from the microtiter plate to the respective pre-coated DA microtiter strips for the DA Elisa test. During this phase, calibrators, controls, and unknowns were incubated with DA antiserum overnight and washed 4 times. The antibody bound to the solid phase was detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate and the reaction was monitored at 450 nm.

2.3. Battery of behavioral tests

Four different cohorts of mice were used in the current study. Each cohort of mice included treated (JNJ injected) and no-treated groups (saline injected). Sucrose preference test, in adolescent mice was studied in the first cohort of mice. The test was performed 24 h after the last administration of JNJ or saline. Open field in adolescent mice and T-maze reversal memory in adulthood were studied in a second cohort of mice, while working and reference memory were tested in a new separate (third) cohort of mice. Open field, social interaction, and sucrose preference, in adulthood, was performed in a fourth cohort of mice. This battery of behaviors was studied in sequential order. Timeline of the behavioral experiments is summarized in Figure 1A.

2.3.1. Sucrose preference test

Mice were single-housed and tested for their preference for sweet solution. In general, mice were given access to two drinking bottles containing tap water for 2 days. On day three, one of the water bottles was replaced with a bottle containing sucrose solution (Sigma, St. Louis, MO) and the consumption of water and sucrose was measured for two or three consecutive days. In adulthood, mice were subject to increasing concentrations of sucrose solution (1%, 1.5%, and 2.5%). Consumption of water and sucrose from each sucrose solution was measured for two consecutive days. The position of the bottles was changed daily to prevent side preference. Results represent the mean of two (adult) or three (adolescence) consecutive days of water or sucrose consumption.

2.3.2. T-maze reversal learning

Mice were maintained on a restricted feeding schedule at approximately 85% of their free-feeding quantity, and habituated to the maze and the reward (Froot Loops, Kellogg Company). The T-maze consisted of a start arm and two identical goal arms with food wells located at the end of each goal arm. Mice were trained to locate a food reward in the goal arm (acquisition phase). In brief, the mouse was first placed in the start arm. The doors to the baited goal and start arms were opened to allow the mouse to walk to the end of the goal arm and to consume the reward. After the mouse returned to the start arm, the door was closed for 5 s, and then reopened to allow the mouse to choose the goal arm to enter (free choice). The mouse was rewarded for choosing the correct goal arm, whereas no reward was given if the incorrect choice (opposite to the goal arm) was made. Each testing trial contained one force run followed by 9 choice runs, and the learning criterion was defined as 80% correct entries for three consecutive days. After learning, the reward goal was switched, and the mice were tested for reversal learning. The number of correct entries and time to reach learning criterion was

Figure 1 Effect of JNJ-28871063 on striatal dopamine levels. (A) Summary of the behavioral experiments timeline. Arrow indicates the starting date of the behavioral task. * and ~ performed in sequential order; separate cohorts were used. (B) Total dopamine levels were measured using dopamine ELISA kit. Dopamine level was 1.7 times higher in JNJ-treated mice than in non-treated controls (n=5 per group). Results represent the mean±SEM, *p<0.05.
scored. Between mice, the arms were cleaned with 70% ethanol to remove olfactory cues.

2.3.3. Radial arms maze (working and reference memory)
Mice were maintained on a restricted feeding schedule at approximately 85% of their free-feeding quantity, and habituated to the maze and the reward (Froot Loops, Kellogg Co.). Following 3 days of habituation, mice were trained to locate food reward at the end of the maze arms. During the test phase only four arms of the eight were baited with reward food. The mice were tested for working and reference memory. Reference memory measures the ability to recall which arms were not baited, while working memory measures the ability to recall which baited arm has already been visited during the daily trial. The sequence of baiting remained constant for each mouse, and animals received one trial per day for 11 consecutive training days. Each daily trial terminated when the four baited arms were chosen and the food collected or 8 min elapsed.

Two types of errors were considered: reference memory and working memory errors. Working memory errors were assessed by scoring the number of repeat entries into a baited arm that had been visited previously. Reference memory was measured by the number of entries into non-baited arms. After the trial of each mouse, the arms were cleaned with 70% ethanol to remove olfactory cues.

2.3.4. Sociability and social memory
Sociability and social memory test was performed according to Silverman et al. (2010). In brief, mice were placed in apparatus divided to three equal chambers. Each trial included a habituation stage of 20 min, followed by two stages. In the first stage (sociability test), an unfamiliar mouse (Stranger 1) was placed in one of the side chambers in a wired cup. An identical empty wired cup was placed in the opposite side chamber, and the subject mouse was allowed to explore the whole apparatus for 10 min. In the second stage (social memory test), a new unfamiliar control mouse (Stranger 2) was placed in the empty wired cup, and the subject mouse again allowed to explore the whole apparatus for 10 min. The amount of time the subject mouse spent in each chamber in each session was recorded and measured automatically with AnyMaze program (Stoeltting Co., Wood Dale, IL). The chamber was wiped between trials with a 70% alcohol solution.

2.3.5. Open field test
Locomotor activity was measured in a 40 × 40 cm² open field arena. Mice were placed in one corner of the arena and their ambulatory activity was recorded for 30 min with AnyMaze program. Measurements of locomotor activity, time spent in the center, and the frequency of visits to the center of the chamber were collected and quantified using the AnyMaze program. The open field chamber was wiped between trials with a 70% alcohol solution.

2.4. Gene expression analysis
Mice were scarified 24 h following the last administration of the JNJ (during adolescence) or following the T-maze reversal test (adulthood), and the frontal cortex was dissected. Total RNA was extracted using TRI reagent (Sigma-Aldrich, St. Louis, MO) according to the manufacturer’s instructions. RNA was reverse transcribed using GoScript Reverse Transcription System (Promega, Madison, WI). Real-time PCR was performed using PerfeCTa Sybr Green FastMix, ROX (Quanta Bioscience, Gaithersburg, MD) and Eco qPCR system (Illumina, San Diego, CA). The thermal cycling program was as follows: hold on 95 °C for 3 min followed by 40 cycles of: 10 s at 95 °C and 60 s at 60 °C. The relative expression of glutamate decarboxylase (GAD) 1 and 2, and DA 2 receptor (D2R) normalized to β-actin, was calculated using the ΔΔCt method. Table 1 lists the primer sequences for the genes examined.

2.5. Statistical analysis
Student’s t-test was used to analyze total DA content, sucrose consumption, weight gain, discrimination and reversal learning, and sociability and social memory. Analysis of sucrose consumption of the adult JNJ-treated group and saline injected group was performed with one-way ANOVA, while two-way ANOVA was used to compare between the two groups. Working memory, reference memory, and exploratory behavior were analyzed using two-way ANOVA.

3. Results
3.1. Effects of blocking the ErbB pathway in adolescent mice
3.1.1. Inhibition of ErbB signaling is associated with an increase in total striatal dopamine
To investigate the role of ErbB signaling in developing cognitive and social skills in mice, we blocked the signaling pathways using the pan-ErbB kinase inhibitor JNJ-28871063 (JNJ) during adolescent brain development, a period when cortical areas involved in decision making, planning, judgments, emotions, and social cognition evolve. We administered 5 mg/kg JNJ i.p. once a day during PND 28-42 (Figure 1A, Spear, 2000). Weight gain over this period was similar for mice administered JNJ and for those who were injected with saline (26.4±0.68 and 26.1±0.48 g, respectively; p>0.05, Student t-test), suggesting that blocking the ErbB pathways has no effect on the growth rate of mice. However, as shown in Figure 1B, total striatal DA was 1.7 times higher in adolescent JNJ-treated mice than in controls (14.6±0.52 and 8.6±1.7 ng/ml±SEM, respectively; p<0.01, Student t-test).

3.1.2. Reduced sucrose preference but not locomotor motor activity was observed in adolescent JNJ treated mice
To explore the functional consequence of elevated DA content we measured the consumption of sweetness solution, and monitored the motor activity and exploratory behavior in the open field. Sweetness consumption was measured using the sucrose preference test over 3 consecutive days. Adolescent JNJ-treated mice did not prefer 1% sucrose solution over water (53±4.5 and 47±4.5%±SEM, respectively; p>0.05, Student’s t-test), while the saline-injected group displayed sucrose preference (73±2.6 and 27±2.6%±SEM, respectively; p<0.00001, Student’s t-test). In addition, JNJ-treated mice consumed

<table>
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<th>Table 1 Primer sequences.</th>
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<tr>
<td><strong>Sequence</strong></td>
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<tr>
<td>GAD 1 Forward: CTCAGGCTGTATGTCAGATTCTC Reverse: AAAGGAGTCAAAGAGATTGTTGTC</td>
</tr>
<tr>
<td>GAD 2 Forward: TCAACTAGTCCCACCCCTAAAG Reverse: CCCCTGATAGTCAATACCTGC</td>
</tr>
<tr>
<td>D2R Forward: ACCGTCCTCTGGATAGTATGAG Reverse: GCATGGCATAGTAGTAGTTGGA</td>
</tr>
<tr>
<td>b-actin Forward: TACTCTGTGTGGATCGGTGG Reverse: GCTCAGTACAGTCCGCCT</td>
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significantly less sucrose than their control group (53 ± 4.5 and 73 ± 2.6% ± SEM, respectively; p < 0.01, Student t-test; Figure 2A). Locomotor activity, habituation to the novel environment, and total distance were not significantly different between adolescent JNJ-treated mice and saline-injected controls, indicating a lack of association between both motor and exploratory behavior, and inhibition of ErbB signaling pathways (Figure 2B and C). Taken together, these results suggest that blocking the ErbB signaling pathway increased total striatal DA content and altered hedonic behavior, but without affecting the motor ability of the adolescent mouse.

3.2. Behavioral analysis of the JNJ-treated mice in adulthood

3.2.1. JNJ-treated mice exhibited reduced unhedonic-like behavior

First we explored whether the impaired reward-seeking behavior (hedonic-like behavior) in adolescence is observed in adulthood. To further explore reward preference we measured the consumption of increasing sweetness-sucrose solution over water. One-way ANOVA analysis revealed that JNJ-treated mice display the same preference for 1.0% , 1.5% , and 2.5% sucrose solutions (1% 51 ± 7 vs. 1.5% 67 ± 9 vs. 2.5% 56 ± 11% ± SEM; p > 0.05; Figure 3), while the saline-injected control group showed greater preference for the increasing sweetness of the solutions over water (1% 55 ± 11 vs. 1.5% 80 ± 5 vs. 2.5% 91 ± 8% ± SEM; p < 0.005). In addition, two-way ANOVA (treatment × solution) detected a statistically significant effect of the treatment (p < 0.05), and post-hoc analysis revealed that the JNJ-treated mice had less preference for 2.5% sucrose solution than did the control group (56 ± 11 and 91 ± 8% ± SEM, respectively; p < 0.05; Figure 3). These results suggest that reduced hedonic drive in adolescence continues throughout adulthood.

3.2.2. Learning and reference memory impaired in JNJ treated mice

The T-maze reversal learning and radial arm maze were used to assay learning and memory (Shoji et al., 2012; Wenk, 2004). In the T-maze reversal learning, the mouse first learns to distinguish between the baited and non-baited arms, and then learns to reverse its choice. During the conditional discrimination learning (acquisition phase),
JNJ treated mice needed significantly more correct-response trials to reach the learning criterion (80% correct entries for three consecutive days) than did the saline injected control group (9 ± 1.3 and 6 ± 0.6 days ± SEM, respectively; p < 0.05, Student's t-test; Figure 4A). However, no differences were observed between the groups in performance on the reversal learning task. Mice who were and were not treated with JNJ reached the learning criterion after 6 days (Figure 4B).

Next, we used the radial arms maze to study working and reference memory. Only four of the eight arms were baited. Therefore, the mouse had to remember which arms were baited, which baited arms it had already entered (working memory), and which arms it should avoid (reference memory). Learning was reflected by the progressive decrease of working memory errors over time, which was similar for both JNJ-treated and control mice (Figure 5A and B). However, two-way ANOVA (treatment × days) analysis of reference memory showed a statistically significant increase of the number of errors among those who received JNJ (p < 0.05; Figure 5A), with no significant interaction with days. No statistically significant differences between control and JNJ-treated mice were observed in the number of working memory errors and in the time to complete the task (Figure 5B and C). These data suggest that blocking the pathway during the cortical maturation period impaired the acquisition of the initial discrimination learning and reference memory, but did not affect working or reversal learning.

3.2.3. JNJ-treated mice display normal sociability, social memory, and exploratory behavior

Social behavior was assayed by the simple sociability and social memory paradigm (Moy et al., 2007). Both JNJ-treated mice and their control group preferred to spend more time in the chamber with the stimulus mouse than in the opposite chamber with the empty cup (Figure 6A; p < 0.0005, Student's t-test). In addition, during the social novelty phase, JNJ-treated mice and their controls spend more time in the chamber with the novel unfamiliar mouse than in the chamber of the familiar stimulus mouse (Figure 6B; JNJ: p < 0.05; Control: p < 0.005, Student's t-test). Both of these effects did not differ between JNJ-treated mice and the control injected group.

Next we re-evaluated the motor activity and exploratory behavior in a novel environment. No significant difference in motor activity and exploratory behavior was displayed in JNJ-treated mice compared with controls (Figure 6C).

3.3. mRNA expression analysis of GAD 1 and 2 and D2R in the medial frontal cortex in adolescence and adulthood

To explore the molecular mechanism underlying the dopaminergic and behavioral changes in JNJ-treated mice, we examined the expressions of GABAergic markers and dopamine receptors in the medial frontal cortex. As summarized in Table 2, analysis of mRNA expression of GAD 1 and 2 and D2R revealed no significant difference in the
expression of the genes between JNJ-treated mice and their injected saline controls in adolescence. However, in adulthood, mRNA expression of D2R was 44% lower in JNJ-treated mice than in controls (0.79 ± 0.19 and 1.76 ± 0.2 A.U ± SEM, respectively; \( p < 0.01 \), Student’s t-test). No significant difference in the expression levels of GAD1 and GAD 2 in adulthood was observed. These results suggest that pharmacologically inhibition of the pathway during adolescence alter the expression of the D2R in the medial prefrontal cortex in adulthood.

4. Discussion

Numerous developmental processes in the central nervous system, including myelination of peripheral nerves, interneuron migration, glutamatergic synapse maturation, synaptic plasticity, and network oscillation, depend upon, or are regulated by, ErbB signaling (Iwakura and Nawa, 2013; Mei and Xiong, 2008). In addition, the signaling pathway has been genetically implicated in schizophrenia (Anttila et al., 2004; Benzel et al., 2007; Harrison and Law, 2006; Lee et al., 2006), and numerous findings support the dysregulation of the NRG and EGF signaling in complex behavior related to schizophrenia and other psychiatric disorders (Chen et al., 2008; Deakin et al., 2011; Duffy et al., 2008; Kato et al., 2010a, 2010b; O’Tuathaigh et al., 2010; Shamir et al., 2012; Sotoyama et al., 2007). However, it is unclear how and at what stage during brain development (prenatal, neonatal or postnatal) the pathway is involved. To further explore the role of the pathway in the neurobiology of schizophrenia, we asked whether inhibition of the pathway during adolescence, a critical period in the development of cognitive and social skills, increase the vulnerability to develop cognitive and social dysfunction in adulthood (Johnson and Wolke, 2013; Novick et al., 2013). In the current study, we are reporting for the first time that inhibition of the pathway during adolescence using a pan-ErbB inhibitor resulted in the unexpected increase of total content of striatal DA in the adolescent mice and in sweetness consumption. In addition, these mice displayed disruption in discrimination learning, reference memory, and hedonic-like (reward-seeking) behavior in adulthood (Table 3). It is important to emphasize that in the current paper the entire ErbB receptor family was pharmacological inactivated. However, the expression of ErbB4 is relatively high at postnatal days and stays stable throughout adulthood, whereas ErbB1 is highly expressed during perinatal development and reducing during postnatal brain development (Abe et al. 2009; Gómez-Pinilla et al., 1988; Mechawar et al. 2007). Thus, the distinct expression patterns suggest that our methodology approach mostly affects the ErbB signaling probably via the ErbB4 receptor, although the IC50 values for ErbB4 and 1 inhibition are the same (21 and 22 nM for ErbB4 and ErbB1, respectively). In stating this, we do not intend to rule out the contribution of other ErbB receptors (i.e ErbB1 or ErbB2).
Acute delivery of NRG1 and of EGF are reported to trigger DA release; therefore, we hypothesize that blocking the pathway would decrease DA levels. Contrary to our expectations based on the aforementioned experiments, we found that blocking the ErbB pathway by systematic injections of the JNJ ErbB blocker during adolescence resulted in increased striatal DA content. However, these observations are consistent with the hyperactivity and reduced pre-pulse inhibition, as well as augmented DA levels (Skirsewski and Buonanno, personal communication), observed in adult ErbB4 null mice where the ErbB system is chronically inactivated. Our counterintuitive result might therefore be explained by the imbalanced action of the ErbB signaling on the dopaminergic system that becomes apparent after prolonged receptor inhibition. The imbalanced action of the pathway suggests that either too little or too much activation of ErbB signaling might increase the release or the amount of DA. As a support for this notion, recently Nawa and his colleagues (Kato et al., 2010a) reported a decrease in DA level in mice overexpressing the NRG1 gene, while other studies reported that exogenous administration of NRG or EGF evokes extracellular DA release (Kato et al., 2010b; Kwon et al., 2008; Yurek et al., 2004). In addition, the ErbB signaling regulates neuronal transmission and excitability, which can disrupt both glutamatergic and GABAergic circuitry (Buonanno, 2010). Since both systems are involved in the regulation of the dopaminergic system (Barrot et al., 2012; Javitt, 2007; Stone et al., 2007), interruption in the GABA- Glutamate balance via inhibition of the ErbB pathway might lead to disruption in the firing of the dopaminergic neurons and to an increase in the DA.

Table 2  mRNA expression levels of GAD1, GAD2, and D2R in the medial frontal cortex.

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<tr>
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<th>Adolescence</th>
<th>Adulthood</th>
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<tr>
<td></td>
<td>Control</td>
<td>JNJ treated</td>
</tr>
<tr>
<td>GAD 1</td>
<td>1.51±0.2</td>
<td>1.61±0.1</td>
</tr>
<tr>
<td>GAD 2</td>
<td>1.06±0.2</td>
<td>1.17±0.4</td>
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<tr>
<td>D2R</td>
<td>2.98±1.3</td>
<td>2.35±0.9</td>
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Results presented in arbitrary units±SEM as relative expression of GAD 1 and 2 and D2R normalized to β-actin. Saline injected mice: n=7 and n=9, JNJ treated mice: n=5 and n=8, adolescence and adulthood, respectively. *p<0.05.
content (Gianutsos and Moore, 1977). As there are many alternative explanations for this controversy, the exact mechanism underlying this phenomenon remains to be explored.

Our data suggest that blocking the ErbB signaling pathway during adolescence induced long-lasting changes in discrimination learning, reference memory, and motivation. Adolescence is a transition period from childhood to adulthood involving complex social, biological, and cognitive changes. Behaviors that include high levels of risk taking, social interaction, decision making, and executive functioning, promote and help adolescents to develop the necessary skills for becoming an independent adult person (Yurgelun-Todd, 2007). During this period particular frontal cortical and subcortical brain regions such as the striatum, thalamus, and nucleus accumbens are structurally remodeled (Blakemore and Choudhury, 2006; Giedd et al., 1999). In particular, changes within cortical and subcortical dopaminergic systems affect the development of cognition and social cognition skills. For example, Naneix et al. (2013) demonstrated that chronic stimulation of D2-type receptors by quinpirole during adolescence alters the development of DA systems, and affects goal-directed behavior in the adult mice. In addition, amphetamine-exposed adolescent rats required more training to reach the learning criterion of delayed nonmatching-to-position tasks in adulthood (Sherrill et al., 2013). Thus, the hyper-dopaminergic state during adolescence might explain some of the behavioral phenotypes observed in the adult JNJ-treated mice. To test our hypothesis, further studies are needed to understand the dynamic effects of pharmacological inhibition of the pathway on the DA system. Furthermore, our results suggest that the pathway may play a role in the development of behaviors that mature during the transition from childhood to adulthood as higher cognitive functions and motivation behavior (Yurgelun-Todd, 2007). Further studies are needed to elucidate the relationship between the ErbB signaling, the dopaminergic system state and the development of anhedonic behavior. Furthermore, our data suggest that impairment of the ErbB signaling pathway in a critical period for the development of motivation and hedonic capacity may trigger the development of one of the key negative symptoms in schizophrenia, reduction in hedonic drive (Pelizza et al., 2012). In addition, these results suggest an alteration in the reward system function, probably by affecting the mesolimbic and mesocortical dopamine pathway and not the nigrostriatal dopaminergic state, since we did not demonstrate changes in locomotor activity in both adolescent and adult mice. Interestingly, reduced hedonic drive and motivation in the JNJ-treated mice have not impaired the social preference and social novelty in adulthood, suggesting that impairment of the pathway during adolescence does not affect social hedonic drive in adulthood. Further analyses of the molecular and signaling processes involved in the ErbB signaling pathway in regulating motivation and hedonic behavior are needed.

In conclusion, our study demonstrated that sub-chronic inhibition of the ErbB signaling during a critical period in developing cognitive and social skills may increase the vulnerability to develop abnormal adult behavior as discrimination learning, motivation and pleasure, and impaired reference memory. In addition, we proposed that at this critical period (adolescence) the dopaminergic system is sensitive to the inhibition of the ErbB signaling pathway. Our finding therefore supports previous work reporting that the ErbB signaling modulates complex behaviors related to schizophrenia and other psychiatric disorders.

### Role of funding source

This work was supported by the National Institute of Psychobiology Young Investigator Research (218-12-13) Grant to A.S. and by the Technion V.P.R. Fund for Medical Research (1011359; A.S.). We are also grateful for the financial support from Ort Bruda College (I.G.).

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### Table 3 Behavioral phenotype summary of JNJ treated mice.

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<tr>
<th>Behavioral test</th>
<th>Adolescent</th>
<th>Adulthood</th>
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<tr>
<td>Sucrose preference test</td>
<td>No preference for sucrose</td>
<td>No preference for sucrose</td>
</tr>
<tr>
<td>Reversal learning</td>
<td>Delay in acquiring the task, but normal reversal learning</td>
<td>Impaired reference memory but not working memory</td>
</tr>
<tr>
<td>Radial arms test</td>
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<td>Normal</td>
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<tr>
<td>Open field sociability and social memory</td>
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ND = not determined.
Contributors

A.S designed this project. I.G. and H.T performed the experiments. A.S wrote the paper, and all the authors discussed the result and commented on the paper.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors thank Timur Livshits, David Helman, Dganit Elbaz and Titana Shreks, Alla Ajamehh, Adham Tarif, Neriman Mattar and Nor Fakhoury for helping with the behavior experiments, and Ms. Edna Oxman for editing the manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2014.09.011.

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