Development of self-regulatory competencies during adolescence is partially dependent on normative brain maturation. Here, we report that adolescent rats as compared to adults exhibit impulsive and compulsive-like behavioral traits, the latter being associated with lower expression of mRNA levels of the immediate early gene zif268 in the anterior insula cortex (AIC). This suggests that underdeveloped AIC function in adolescent rats could contribute to an immature pattern of interoceptive cue integration in decision making and a compulsive phenotype. In support of this, we report that layer 5 pyramidal neurons in the adolescent rat AIC are hypoexcitable and receive fewer glutamatergic inputs compared to adults. Chemogenetic activation of the AIC attenuated compulsive traits in adolescent rats supporting the idea that in early stages of AIC maturity there exists a suboptimal integration of sensory and cognitive information that contributes to inflexible behaviors in specific conditions of reward availability.

Compelling evidence suggests that heightened sensation-seeking, together with risk taking and reckless behaviors, are a major cause of morbidity and mortality among teenagers (1, 2). Clinical studies also suggest that adolescents engage in dangerous activities despite knowing and understanding the risks involved (3), emphasizing that adolescents remain vulnerable to impulsiveness (i.e., acting prematurely without adequate forethought) due to incomplete development of executive cognitive functions (planning, abstract reasoning, and response inhibition) (4). Therefore, adolescence is not only a transition phase from childhood to adulthood but a normative process defined by the emergence of a sagacious mind shaped by the intricate influence of multiple experiences made of social pressure and adjustments in personal goals. Meanwhile, teenagers often display careless behaviors, particularly when acting recklessly is perceived as necessary for increased peer recognition (3, 5, 6). However, thrill-seeking in adolescence should not necessarily be perceived as a negative developmental trait but one that can also be considered instrumental in shaping the adolescent brain to develop cognitive control through multiple experiences (7). As the underpinnings of adolescent risk-taking behaviors are poorly characterized, knowledge of the neurobiological mechanisms involved in this developmental stage is important to better define this transformative phase of brain circuitry.

Near the beginning of adolescence, the brain undergoes several structural and network reorganizations (8, 9). The discrepant trajectories of development characterizing the adolescent brain have engendered several theories of increased adolescent risk-taking (3, 10–13) that share a common core of the asynchronous development of neural systems underlying reward-seeking and self-regulation (14). The limbic system matures earlier than the prefrontal cortex (PFC) and could be one reason for increased reward sensitivity resulting in increased sensation-seeking and self-regulation (15). It is argued that the delayed maturation of the PFC may represent increasing, but still incomplete, frontal control over behavior during adolescence (19, 20), gradually facilitating cognitive capacities for risk assessment (14, 21–24). Whereas the mechanisms by which self-regulatory and affective brain networks interact remain unclear, converging evidence suggests that the insular cortex is critical for emotion regulation, cognitive control, and ultimately flexible behavior (25–29). Therefore, the tendency of adolescents to engage in risky behavior may also be due to their inability to engage harm avoidance circuitry including the anterior insular cortex (AIC) during decision making (30). Within this perspective, extensive review of the literature suggests that the key role of the AIC is in the integration of top-down cognitive predictions and bottom-up interoceptive signals for emotional awareness (31), contributing critically to the cognitive control network implicated in the coordination of thoughts and actions (32). In other words, the most adaptive behavioral response is predicated upon the integration of

Significance
Clinical evidence suggests that adolescents engage in dangerous activities despite understanding the risks involved, questioning the theory of decreased top-down control of the immature prefrontal cortex promoting adolescent disinhibited behaviors. In the present study, we report that adolescent rats show a much higher degree of inflexible behavior when making decisions under conflict compared to adults. Unexpectedly, we identified a lower excitability of layer 5 pyramidal neurons in the anterior insular cortex (AIC) of adolescent rats and smaller synaptic glutamatergic inputs to these cells but no difference in layer 5 prefrontal cortex pyramidal neurons. Chemogenetic activation of AIC neurons reduced persistent reward-seeking despite punishment, suggesting that the delayed maturation of the insula may promote inflexible reward-related behaviors in adolescent rats.
Results

Adolescent and Adult Rats Display Similar Behavioral Responses in the Absence of Conflictual Decision Making.

Since the AIC is considered an integrative hub that coordinates the recruitment of task- and context-relevant brain networks as a response to arousal (28), we conducted a series of behavioral experiments in adolescent and adult rats to assess their emotional coping strategies (35). First, adolescent (postnatal day [PND] 40–50) and adult (PND 90–100) rats exhibited comparable locomotor activity in an open field (Fig. 1A) and manifested equal preference for a novel environment (Fig. 1B), suggesting a similar emotional response and exploratory behavior to an unknown but neutral environment. We then challenged them in conditions of stress-induced arousal. In the passive avoidance paradigm, both groups similarly increased the latency to enter a dark compartment after previously experiencing mild electrical foot shocks in this environment (Fig. 1C). In the contextual fear conditioning procedure, adolescents exhibited a transiently enhanced freezing response after initial exposure to electrical foot shocks but normalized over time within the session compared to adults (Fig. 1D). Finally, an enhanced emotional response was found in adolescent rats exploring the elevated plus maze, reflected in the reduced exploration of the open arms compared to adults (Fig. 1E), despite similar levels of locomotion. Overall, exploratory behaviors in stressful environments differed only moderately between adolescent and adult rats.

We then assessed adaptive behavioral coping strategies as a possible measure of executive control and reward sensitivity (35) using operant conditioning paradigms. We first observed a similar capacity to self-control in the absence of reward availability after extended training for saccharine self-administration. Specifically, although adult rats exhibited greater responding than adolescents on the active lever for reward when saccharine was available, most likely reflecting their higher ingestion capacity, both groups significantly decreased their reward-seeking behavior during periods of signaled unavailability (Fig. 1F). Ultimately, response inhibition (reflecting the drastic decrease in lever presses when reward is no longer available) was similar between groups emphasizing that adolescent and adult rats exhibited comparable levels of response inhibition in the signaled absence of reward (Fig. 1G). In addition, adolescent and adult rats displayed a similar level of motivation during progressive ratio schedules of reinforcement as indicated by the total number of lever presses (Fig. 1H) and the total number of rewards earned (Fig. 1A).

Overall, these observations indicate that adolescent and adult rats manifest generally comparable general coping strategies in the absence of conflictual decision making, and that adolescents exhibit larger fear responses when presented with a physical threat (foot shock).

Adolescent Rats Display Persistent Aberrant Reward Taking Behaviors in Conflictual Situations Reflecting Enhanced Impulsive- and Compulsive-Like Behaviors.

Converging evidence suggests that the maturation of brain regions supporting response inhibition may underlie the decline in risk taking behavior observed from adolescence through adulthood (4). Here, we first tested adolescent and adult rats in a 5-choice serial reaction time task to assess their response inhibition capacity when sucrose food pellet delivery depends on behavioral performance contingent on stringent rules where withholding the behavioral response is mandatory and breaking the rule is counterproductive. Rats were trained and ultimately tested while food restricted and tested again while fed ad libitum. After extensive training sessions, adolescent and adult rats exhibited comparable performance with regard to response accuracy (Fig. 1J) and the number of omissions (Fig. 1A), whether satiated or not. In contrast to this behavior, adolescent rats made more premature responses than adults, and this was enhanced when rats were tested under food-restricted conditions (Fig. 1L). Thus, adolescent rats expressed significantly higher premature responses compared to adults upon food restriction, but this was not observed when rats were fed ad libitum (two way RM-ANOVA, group × food restriction interaction, F[1,21] = 4.78, P = 0.04). These observations suggest that in a conflictual situation (withholding a behavioral response while hunger promotes food-seeking), adolescent rats encounter greater difficulty to adapt and manifest a counterproductive impulsive-like response.

Following these observations, we further challenged adolescent and adult rats using a conflict paradigm in which reward delivery was followed by punishment. Here, rats fed ad libitum had to adapt their lever pressing-behavior when saccharine delivery was followed by an electrical foot shock that increased in intensity over three consecutive sessions. As expected, lever presses decreased with increased shock intensity from 0.22 to 0.33 mA in all rats, but to a significantly lesser extent in adolescents. Strikingly, the adolescents persisted in lever pressing despite the 0.22 mA mild electrical foot shock, suggesting a compulsive-like reward-seeking behavior (Fig. 1M). Importantly, this effect could not be attributed to differences in nociceptive thresholds between adolescent and adult rats (SI Appendix, Fig. 1).

Lower Functional Recruitment of the Anterior Insula Cortex in Adolescent Rats Compared to Adults.

To investigate potential mechanisms of differences in compulsive responding between adult and adolescent rats we examined the expression of mRNA for the transcription factor zif268 in the prelimbic cortex (PLC)
Adolescent and adult rats display similar behavioral responses in absence of conflictual decision making: measures of emotional reactivity. (A) Locomotor habituation: adolescent (n = 9, PND 50) and adult (n = 7, PND 90) rats exhibited a similar locomotor activity in an open field (two-way repeated measures ANOVA, F[3,42] = 24.47, P < 0.0001, reflecting a common habituation to the arena over time, with no group effect [F[1,14] = 0.74, P = 0.788], and no group × time interaction [F(3,42) = 0.77, P = 0.51]. (B) Novelty preference: adolescent (n = 9, PND 50) and adult (n = 6, PND 90) rats also manifested the same attractiveness for a novel environment (Mann-Whitney test, U = 20, P = 0.48) suggesting similar emotional response and exploratory behavior to an unknown but neutral environment. (C) Passive avoidance: adolescent (n = 9, PND 50) and adult (n = 9, PND 90) rats demonstrated similar increases in latency to enter the dark compartment after experiencing mild electrical foot shocks in this environment (two-way repeated measures ANOVA, F[2,32] = 40.58, P < 0.0001; no group effect, F[1,16] = 1.623, P = 0.22; and no interaction, F[2,32] = 1.026, P = 0.37). (D) Fear conditioning: adolescents (n = 10, PND 50) transiently exhibited an enhanced freezing response after the first exposure to electrical foot shocks (two-way repeated measures ANOVA, F[1,17] = 12.02, P = 0.003); post hoc Sidak’s test P < 0.05; no interaction, F[468] = 0.84, P = 0.5), but ultimately expressed the same behavioral response compared to adults (n = 10, PND 90) (group: t[17] = 1.84, P = 0.08, unpaired t test). (E) Elevated plus maze: adolescents (n = 11, PND 50) exhibited a reduced exploration of the open arms compared to adults (n = 14, PND 90) (time spent on open arms: t[23] = 2.36, P = 0.026, unpaired t test) despite similar exploratory behaviors (locomotor activity on closed arms: t[23] = 0.15, P > 0.05; locomotor activity on open arms: t[23] = 0.34, P > 0.05, unpaired t tests) (SI Appendix, Material and Methods). Adolescent and adult rats display similar behavioral responses in absence of conflictual decision making: measures of executive control and reward sensitivity. (F) Control over reward-seeking: adolescent (n = 29, PND 50) rats exhibited a decreased lever-pressing behavior for saccharine compared to adults (n = 29, PND 90) (two-way repeated measures ANOVA; group effect, F[1,56] = 15.42, P = 0.0002; reward availability effect, F[1,56] = 505, P = 0.0001; and groups × reward availability interaction effect, F[1,56] = 15.24, P = 0.0003). Post hoc comparisons confirmed that adult rats were more active than adolescent rats when saccharine was available (Sidak’s multiple comparisons test t[11]2 = 5.524, P < 0.0001), but not during periods of reward unavailability (Sidak’s multiple comparisons test t[11]2 = 0.405, P = 0.9). (G) Control over reward-seeking response inhibition: response inhibition (defined as the ratio of active lever presses during reward unavailability × 100/total active lever presses) was identical between groups (Mann-Whitney test, U = 330, P = 0.16) emphasizing that adolescent and adult rats exhibited similar self-control over “unavailable reward-seeking”. (H) Progressive ratio – breaking point: adolescent (n = 29, PND 50) and adult (n = 29, PND 90) rats displayed a similar motivation on an effort demanding reward-seeking task as indicated by the total number of lever presses (unpaired T test, t[56] = 0.82, P = 0.41). (I) Progressive ratio – rewards earned: the total number of rewards earned (unpaired T test, t[56] = 0.92, P = 0.35). Adolescent rats display persistent aberrant reward-seeking behaviors in conflictual situations reflecting enhanced impulsive- and compulsive-like behaviors. (J) SCSRTT-accuracy: adolescent (n = 12, PND 50) and adult (n = 11, PND 90) rats displayed similar response accuracy, food restricted or not (two-way repeated measures ANOVA; no group effect, F[1,21] = 2.29, P = 0.14; no food restriction effect, F[1,21] = 1.3, P = 0.26; and no group × food restriction interaction, F[1,21] = 2.9, P = 0.103). (K) SCSRTT-omissions: they also displayed similar percentage of omissions (two-way repeated measures ANOVA) across groups; significant group effect, F[1,21] = 29.15, P < 0.0001; significant food restriction effect, F[1,21] = 45.55, P < 0.0001; and significant group × food restriction interaction, F[1,21] = 4.78, P = 0.04). Post hoc Sidak’s test, adolescent rats expressed higher premature responses compared to adults upon food restriction (t[42] = 5.33, P < 0.0001) but not during fed ad libitum state (t[42] = 2.2, P = 0.065). Data represent an average of three consecutive sessions, in food-restricted and food-ad libitum state. (M) Compulsivity paradigm: finally, adolescents (n = 39, PND 50) and adult (n = 28, PND 90) rats displayed increased compulsivity reflected by the higher number of lever presses despite foot shocks (two-way repeated measures ANOVA; significant group effect, F[1,63] = 11.58, P = 0.0011; significant shock intensity effect, F[2,130] = 58.92, P < 0.0001; and significant group × shock intensity interaction, F[2,130] = 6.126, P = 0.0029). Post hoc Sidak’s assessment indicated that adolescent rats significantly persisted in lever pressing despite electrical foot shock intensity of 0.22 mA as compared to adults (t[195] = 4.83, P < 0.0001).
glutamatergic inputs impinging on these cells was assessed using in vitro whole-cell recordings. Pyramidal neurons were identified using electrophysiological criteria, such as the lack of spontaneous action potentials after gaining whole-cell access, and the presence of action potential accommodation upon current injection.

We found that rheobase (Fig. 2B) and input resistance (Fig. 2C) of L5 PLC pyramidal neurons did not significantly differ between adult and adolescent rats. Moreover, these neurons also exhibited similar action potential discharge rates in response to increasing current injections (Fig. 2D), and the mean amplitudes of electrically evoked excitatory postsynaptic currents (eEPSCs) did not differ between adolescent and adult rats (Fig. 2E). Interestingly, however, a smaller paired pulse ratio at interstimulus intervals of 50 ms and 75 ms was observed in the adolescent group indicating a possible decrease in the probability of glutamate release from the presynaptic projections on to the PLC L5 pyramidal neurons (Fig. 2F).

Like the PLC, AIC L5 pyramidal neuron input resistance did not differ between adult and adolescent rats (Fig. 3B). However, unlike the PLC, the rheobase of the AIC L5 pyramidal neurons was significantly higher in the adolescent group as compared to adults (Fig. 3C). Consistent with this higher threshold for excitability, AIC L5 neurons from adolescent rats displayed reduced firing frequency in response to increasing current injections (Fig. 3D). We also found that the relationship between stimulus intensity and eEPSC amplitude was significantly smaller in adolescent AIC L5 pyramidal neurons, compared to adults (Fig. 3E). Moreover, smaller paired pulse ratios were observed in L5 pyramidal neurons from adolescents at interstimulus intervals of 50 ms and 75 ms, suggesting a reduced probability of glutamate release from the axonal projections onto AIC L5 pyramidal neurons in the adolescent group (Fig. 3F).

**Chemogenetic Activation of the AIC Attenuates Compulsive-Like Behavior in Adolescent Rats.** As our electrophysiological studies indicated that L5 AIC neurons were hypexcitable and received less excitatory input in adolescent rats, we hypothesized that bilateral chemogenetic activation of the AIC using the hM3D construct would decrease foot shock resistant reward taking in adolescent rats (Fig. 4A and B). We found that the DREADD agonist clozapine had no effect on basal performance either in sham adolescent controls or in rats injected with AAV-hM3D on PND 45–46 (SI Appendix, Fig. 2).
However, clozapine injection resulted in a significant reduction of saccharine taking during punished responding in the adolescent rats that had received AIC injections of the excitatory DREADD viral construct (Fig. 4 C and D).

Altogether, these results indicate that chemogenetic reversal of AIC hypoexcitability in juvenile rats does not disrupt basal performance in an operant conditioning task in the absence of foot shocks, but significantly reduces persistent lever-pressing behavior during conflictual decision making, when a reward is paired with a punishment.

**Discussion**

In the present experiments, we found that whereas adolescent rats exhibited many of the general coping strategies present in adults, they also showed a much higher degree of inflexible behavior when making decisions during conflict demands. We also observed a decrease in mRNA expression of zif268 in the AIC of adolescent rats as well as a lower intrinsic excitability of L5 pyramidal neurons together with weaker glutamatergic synaptic input to these cells. To determine whether this functional immaturity of the AIC could underlie increased compulsive behavior in adolescent rats, we reversed the hypoexcitable state of the AIC by chemogenetic activation and found that this significantly attenuated the compulsive behavior.

According to neural imbalance models of behavioral control, top-down inhibition of subcortical brain structures by cortical regions is hypothesized to be weak during adolescence, promoting impulsivity, sensation-seeking, and reckless behavior (4, 6). However, it is also thought that increased risk and novelty-seeking during maturation may be advantageous to facilitate novel learning strategies through this behavior (6).

Here, we found that adolescent and adult rats largely exhibited similar open field exploration and novelty place preference behavior (Fig. 1 A and B) (4, 36–40). They also displayed a similar drive to explore a novel environment, comparable abilities to inhibit saccharine seeking in its signaled absence (Fig. 1 F and G), and comparable learning skills in our motor
impulsivity paradigm (Fig. 1J and K). However, adolescent rats made more premature responses than adults in the 5CSRTT during food restriction. This observation suggests the inability to self-regulate under a conflicted situation of increased drive to gain food, as reported previously (36, 41, 42). It also confirms that the ability to regulate reward and sensation-seeking behaviors may not depend solely on impulse control but may rely on the integration of sensory and

**Fig. 4.** Chemogenetic stimulation of the anterior insular cortex attenuates compulsive-like behavior in adolescent rats. (A) Timeline of the chemogenetic experimentation. (B) Representative immunofluorescence image showing the expression of AAV8 excitatory DREADD (pAAV-CaMKIIa-hM3D(Gq)-mCherry) injected in the anterior insula cortex (right hemisphere). (C1) Chemogenetic activation of the anterior insula cortex (mean and individual values): On the test day, rats were injected with either clozapine (0.1 mg/kg, intraperitoneal [i.p.]) or vehicle (equivalent volume, i.p.) 30 min prior to the test following a Latin square design. A two-way repeated measures ANOVA revealed that hM3D virus injection in the AIC had no effect on rats performance (group effect, \(F_{[1,14]} = 0.049, P = 0.82\)) and treatment had globally no effect as well (treatment effect, \(F_{[1,14]} = 0.0027, P = 0.95\)), but a significant interaction effect was observed (interaction, \(F_{[1,14]} = 7.653, P = 0.01\)) emphasizing a specific effect of clozapine in rats having received the hM3D virus. A post hoc simple effect analysis confirmed no effect of clozapine in the Sham surgery group (\(t_{[8]} = 1.67, P = 0.13\)), but a significant decrease in the total number of lever presses in rats injected with hM3D virus following clozapine injection (\(t_{[6]} = 3.219, P = 0.018\)). See SI Appendix, Fig. 2 for further information. (C2) Estimation plot using the metric (number of lever presses) as the left y axis and the mean difference with 95% confidence interval (CI) as the right y axis indicates the robust effect of chemogenetic activation of AIC pyramidal neurons on compulsive behavior in adolescent rats. (D1) Chemogenetic activation of the anterior insula cortex; lever-pressing behavior adjusted for baseline performance. Two-way repeated measures ANOVA; no group effect, \(F_{[1,14]} = 0.67, P = 0.42\); significant clozapine injection effect, \(F_{[1,14]} = 5.167, P = 0.039\); and no group \(\times\) clozapine injection effect, \(F_{[1,14]} = 3.114, P = 0.099\). A planned comparison post hoc Sidak's test shows a significant decrease in lever-pressing behavior following clozapine induced activation of the AIC in rats injected with Gq virus (\(P = 0.0348\)), no effect of clozapine injection in the sham surgery group (\(P = 0.91\)). (D2) Estimation plot graphic using the metric (percentage of baseline lever-pressing behavior) as the left y axis and the mean difference with 95% CI as the right y axis indicates the robust effect of chemogenetic activation of AIC pyramidal neurons on compulsive behavior in adolescent rats.
cognitive processes. Supporting this interpretation, adolescent rodents exhibit better performance when seeking rewards in a cue-guided reversal procedure (with location of reward indicated by odors) (43). They also perform better in tasks revealing cognitive flexibility through different reward contingencies (44). This enhanced flexibility in adolescence, considered as a PFC-dependent function (45, 46), is rather counterintuitive to the idea that the PFC is considered functionally immature at this developmental stage and calls for reassessment of current interpretations of the involvement of this brain structure in these behaviors (3, 6, 10, 12).

A key observation in our study is that adolescent rats exhibit higher persistence for saccharine reward despite negative consequences (Fig. 1M), whether this distinct behavior between adolescent and adult rats may reflect differences in fear processing or differences in instrumental learning remains a matter of debate since it was not explicitly explored in this experiment, it could be potentially explored in future studies. Furthermore, this observation is in line with the inability to self-regulate under a conflicted situation as described above. Many clinical and preclinical studies have indeed reported that impulsivity is an endophenotype for higher vulnerability to compulsive behavior (29, 47–52), potentially reflecting a loss of control over reward-seeking in rodents (53, 54) and enhanced risk-taking in humans (55, 56). In the current study, we controlled for potential differences in the appetitive properties of saccharine (Fig. 1L and M) and pain thresholds (SI Appendix; Fig. 1) between adolescent and adult rats. We also controlled for emotional reactivity to pain or risky situations in adolescents, and report similar passive avoidance, increased freezing behavior and increased time in the closed arm of the elevated plus maze (Fig. 1 C–E). Thus, this mitigates the idea that differences in fear processing could explain higher risk taking in adolescent rats. We also show similar mRNA expression of the early gene marker zif268 in the PLC (Fig. 2A) and confirmed similar intrinsic excitability of L5 PLC pyramidal neurons in adolescents and adults (Fig. 2 B–E). In contrast to our results with PLC, we found decreased expression of zif268 mRNA in AIC, and this was accompanied by a lower excitability of AIC L5 pyramidal neurons and weaker synaptic glutamatergic inputs to these neurons in adolescent rats (Fig. 3 B–E). This indicates that not only do these neurons receive weaker glutamatergic projections, but that they are also less responsive to these inputs, reflecting reduced ability to integrate information. Thus, our results confirm that increased sensitivity to punishment from adolescence to adulthood may be observed by greater neural recruitment of the insula (30). Considering that both the rodent medial PLC and primate dorsolateral PFC support executive functions (57), our observation challenges the theory of decreased top-down control of the immature PFC as a possible explanation for adolescent compulsivity and supports the idea that the AIC plays a larger role in this regard.

Our identification of the relationship between neuronal hypoexcitability of the AIC and increased compulsive behavior in adolescent rats is correlational. Therefore, we used chemogenetics to determine whether reversing the AIC hypoactivity in adolescent rats during punished reward taking could alter this behavior. We found that chemogenetic stimulation of the AIC in adolescent rats significantly attenuated the persistent responding for reward during foot shock punishment in adolescent rats (Fig. 4) without affecting motor performance (SI Appendix; Fig. 2). This suggests that the diminished excitability of AIC L5 pyramidal neurons and the smaller synaptic glutamate drive of these cells likely contribute to differences in AIC-dependent behavior between adolescent and adult rats. It may be possible that chemogenetic stimulation of the AIC might disrupt the gustatory reinforcing properties of saccharine, rather than restoring decision making under conflict situation. However, this would necessarily also decrease the motivation for reward-seeking and this was not observed when the AIC was chemogenetically stimulated with clozapine in the absence of a punishing stimulus (SI Appendix, Fig. 2). Moreover, the adjacent orbitofrontal cortex, which is involved in compulsive behaviors (58), was not affected by viral infusions targeting the AIC (SI Appendix, Fig. 3). Therefore, our control data indicate a specific role of the AIC in the manifestation of compulsive behavior and suggest that AIC activation does not alter reward salience per se.

The decreased synaptic glutamatergic strength together with the hypoexcitability of AIC pyramidal neurons could represent a mechanistic explanation for the lower ability of adolescent rats to integrate sensory, emotional and cognitive information (27, 28), and this might explain why adolescent rats are less able to process converging interoceptive signals, particularly aversive ones during risky decision making tasks, as has already been suggested in humans (59–61). Moreover, the decreased functional activity in AIC in contrast with PLC in adult and adolescent rats may reflect the asynchronous maturation of these cortical structures, suggesting also that the connections between these two structures are not fully established in adolescent rodents.

A recent hypothesis suggests that the insula computes interoceptive predictions by estimating both current and future physiological needs (i.e., a change in an expected interoceptive state) to guide brain and behavior toward a homeostatic set point (62). In this model, rather than the simple integration of sensory inputs, interoceptive perceptions can be viewed as a Bayesian estimation of past experience (inference) about the sensory consequences of homeostatic budgeting that are implemented as upcoming visceromotor signals (62, 63). These inferences are also constrained by error signals that result from the failure of previous predictions to accurately account for incoming interoceptive sensations (64). Ultimately, not only does past visceral-sensory experience influence the present experience, but the present one projects forward to influence what will be perceived in the future; meaning that interoceptive perception is largely constructed of beliefs constrained by the actual state of the body (65). This fundamental assumption supports the idea that the insula makes interoceptive predictions and computes prediction errors that could be considered risk prediction and risk prediction errors. Supporting this interpretation, a late-onset anticipatory risk prediction signal followed by a fast-onset prediction error signal at the time of the outcome has been reported in AIC during a gambling task in humans (66). Considering this bimodal response within the AIC for prediction learning, the long-lasting effect of the chemogenetic activation we report here might have partially biased the expected substantial lowering of compulsive lever pressing in adolescent rats (Fig. 4). Another comment arising from this assumption regarding the need to minimize the difference between the brain’s prediction and incoming sensation is the issue of how and when specific bodily signals are consciously represented in adolescent and adult brains. The neuronal hypoexcitability reported here in the AIC of adolescent rats opens an interesting debate about how the risk is integrated (awareness) but not learned optimally (consciousness) (26), perhaps explaining the persistent lever pressing despite punishment observed in adolescents. Considering this transient functional anosognosia (that
we would define as an operating self-awareness but not yet a full consciousness), we would like to suggest that the maturation of the ACC contributes to the construction of brain positive (and negative) alliesthesia (67) in the adolescent brain. In line with the role attributed to the ACC in the active inference framework, perception and action are tightly coupled, according to brain’s estimations following the integration of both extero and interoceptive cues. Interoceptive perceptions therefore derive from the brain’s best prediction to infer the causes of the sensations it receives, constrained by incoming sensory inputs. Either the adolescent brain accepts the punishment as a prerequisite for accessing the reward when the adult considers it is not worth the behavioral output, or the anticipated lack of reward is integrated as an even more painful punishment as compared to the foot shock itself, and the persistent lever-pressing behavior is motivated by the expected positive affective state associated with reward consumption. Our observations only partially unveil how the delayed maturation of the insula may result in suboptimal integration of sensory and cognitive information in adolescents, possibly promoting inflexible reward-related behaviors. Understanding how interoceptive inference contributes to shaping the emotional brain, notably during adolescent development, is of the highest relevance, in particular for preventing the emergence of psychiatric conditions.

Material and Methods

Animals. All experiments conducted in Switzerland used male Wistar rats (130 adolescents and 102 adults) from our breeding colony (breeders ordered from Charles River, France). Adolescent rats (PND 21) weighed ~60-80 g and adult rats (PND 70) weighed 250–300 g at the beginning of experiments. For the electrophysiology experiments, six adolescent and six adult male Wistar rats were ordered from Charles River, USA. Rats were grouped three per cage (560 × 330 × 270 mm). The electrophysiology experiments were conducted at PND 50 for adolescent rats and PND 90 for adult rats.

Adolescent and adult rats were tested on behavioral tasks involving expression of spontaneous behavior in the absence of conflicted decision making such as open field locomotor activity, novelty preference, active and passive avoidance, elevated plus maze performance, reward-seeking in the absence of availability, and motivation for reward taking in the progressive ratio operant task. Both group of rats were also tested on the behavioral tasks involving decision making in presence of conflicted situations such as the 5-choice serial reaction time task (5CSRRT) that tests motor impulsivity, and in the persistence in reward-taking in presence of foot shocks to examine compulsive reward-seeking behavior.

The functional recruitment of the prelimbic and anterior insular cortex was tested by measuring the mRNA levels of zif268 using quantitative polymerase chain reaction immediately after the compulsivity task in both groups. The intrinsic excitability as well as the strength of the glutamatergic inputs received by the layer 5 pyramidal neurons in prelimbic and anterior insular cortices was investigated using in vitro electrophysiology.

Finally, in other groups of adolescent rats, an AAV8-coupled excitatory DREADD or an inactive shamt construct was injected in the anterior insular cortex (AP: +2.4, ML: +4, DV: −5.2). Rats were then evaluated using a Latin square design (to determine clozapine to activate the excitatory DREADD or vehicle injections as a control) for the persistence of reward-taking in the presence of foot shock.

More detailed behavioral, molecular, electrophysiology, and chemogenetic experimental procedures are explained in the SI Appendix, section.

Statistical Analyses. The data are represented as group mean ± SEM. For single group comparison, Student’s t-tests were performed for normally distributed data, or Mann-Whitney U tests were used for nonnormally distributed data. The ANOVA was used for repeated measurements, with nonlinear mixed effect models applied to deal with missing values or nonnormal data distribution. Post hoc Sidak’s tests were used for identifying group level differences. All statistical analyses were conducted using GraphPad Prism (version 6.07). Significance of results was accepted at P < 0.05.

Data Availability. Raw data have been deposited in Zenodo, https://doi.org/10.5281/zenodo.6533820 (68).

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